Pathology of co-contamination of mycotoxins in poultry farms of Aizawl district of Mizoram

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ABSTRACT

The study was conducted to investigate the naturally occurring pathology of co-contamination of mycotoxins in poultry farms in Aizawl, Mizoram. During the investigation, different chicken farms (n=73) were surveyed for occurrence of disease and mortality. The feed samples collected from affected farms were tested for the presence of aflatoxin, ochratoxin and zearalenone by ELISA kits. The dead birds were subjected to necropsy. Visceral organs from the dead birds were processed for histopathological studies. Fourteen feed samples, out of 49 tested (28.5%) were found affected by co-contamination with aflatoxin, ochratoxin and zearalenone. In this study, the mean level of aflatoxin, ochratoxin and zearalenone was 13.49693 ppb, 4.296286 ppb and 81.74543 ppb, respectively. Clinical signs were ruffled feathers, depression, dullness, huddling, poor growth, anorexia. Necropsy revealed pathological lesions in visceral and lymphoid organs. Histopathological findings were inflammations, degenerative, and necrotic lesions in liver and kidneys. A perusal of available literature did not reveal any study on the presence of co-contamination of Mycotoxicosis in poultry and poultry feed in Aizawl.

Keywords: Aflatoxin, Aizawl, ELISA, Ochratoxin, Zearalenone

Mycotoxicoses, resulting from mycotoxin poisoning by fungi such as Aspergillus, Fusarium, and Penicillium, commonly occur in poultry due to natural contamination of raw feed ingredients (Khan et al. 2011). Poultry are highly susceptible to major mycotoxins, such as aflatoxins, ochratoxins, trichothecenes, zearalenone, oosporein, citrinin, ergot alkaloids, and fumonisins, particularly aflatoxins and ochratoxins, leading to mycotoxicoses (Anjum et al. 2011). Aspergillus flavus, Aspergillus parasiticus, and Penicillium puberulum produces the six major aflatoxins: B1, B2, G1, G2, M1, and M2. Out of these, aflatoxin B1 (AFB1) stands out to be the most toxic among them, possessing carcinogenic properties and primarily targeting the liver as its organ of toxicity. Ochratoxins are produced by Aspergillus and Penicillium (Reverberi et al. 2010) and the feed contaminated by ochratoxin leads to development of renal diseases. On the contrary, Zearalenones produced by Fusarium graminearum, Fusarium roseum, exhibits estrogenic activity. While it is relatively less toxic to chickens, it serves as an indicator for the potential presence of several other active toxins. Ochratoxicosis occurrences, often cocontaminant with other mycotoxins like aflatoxin, have been reported worldwide. Parvathi et al. (2017) reported

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Aflatoxin contamination of feed in Warangal, Andhra Pradesh. Kalita *et al.* (2021) reported contamination of various poultry ration with aflatoxin in Aizawl, Mizoram. Park *et al.* (2005) reported contamination of zearalenone with ochratoxin, aflatoxin, DON and nivalenol in rice samples collected from Korea.

Chicken is the predominant domesticated poultry bird in India, and while poultry farming was traditionally a backyard activity until 1960s, the landscape has transformed significantly in the past few decades. India's poultry sector, totaling 851.81 million birds (Annual Report 2022-2023), is rapidly expanding. The North-Eastern hill region (NEHR) of India is predominantly inhabited by non-vegetarians from various tribal communities. Consequently, there exists a substantial demand for meat and eggs in this area. Mizoram has experienced notable advancements, shifting from traditional to intensive commercial poultry farming in the past decade. Despite this growth, challenges such as diseases, feed storage, climate conditions, vaccine failures, mycotoxicosis impact the poultry sector. Even with these known risks, there is a literature gap on the combined toxicity of aflatoxin, ochratoxin and zearalenone in poultry feeds, especially in the North-Eastern region of India. Therefore, the current study was designed considering immuno-detection, occurrence and pathology associated with mycotoxin contamination (aflatoxin, ochratoxin and zearalenone) in feeds utilized in various chicken farms of Aizawl.

MATERIALS AND METHODS

The present study was conducted with a non-random sampling method in rural parts of Aizawl district of Mizoram. The farms under the study consisted of flock sizes varying from 14 to 80 birds. The study was directed towards those farms which revealed episodes of diseases and mortality. Based on pathological lesions observed in the dead birds, the feed samples from the affected farms were collected and tested. During the study period, a total of 73 chicken farms underwent investigation. These farms were consistently visited and observed for disease episodes and mortality. Clinical observations were documented, and dead birds were subjected to necropsy examination and further histopathological examination. Feed samples were collected for ELISA (enzyme-linked immunosorbent assay) based estimation of various mycotoxins.

Clinical signs shown by the affected birds were recorded. The dead birds which were not showing advanced putrefactive changes were collected in chilled containers and immediately transported to the laboratory for a necropsy to observe the gross lesions of various organs especially the liver and kidneys. The necropsy examination was performed immediately on receipt of the bird carcasses. All the collected dead birds were subjected to necropsy examination at the Department of Veterinary Pathology of College in Selesih, Aizawl. The standard procedure of necropsy examination was followed, and pathological lesions were observed, recorded, and photographed. Representative tissue samples from the targeted organs showing gross lesions were collected in 10% neutral buffered formalin (NBF) for fixation and further histopathological analysis. Formalin-fixed tissue pieces were processed for routine histopathology by paraffin embedding and hematoxylin & eosin (H&E) staining Bancroft and Gamble (2008). Procedures of Special Staining Luna Ishak staining and Masson's Trichrome stain were done to demonstrate bile canaliculi and demonstration of collagen fibers in the liver samples respectively Luna (1968).

Total aflatoxin, ochratoxin and zearalenone in feed samples was estimated using a commercially available competitive ELISA kits (TecnaSrl, Italy; Catalogue code-MA210/ MA211, OR360/OR361, & MZ670-MZ671). Following preparation of samples, the ELISA procedure was performed according to the manufacturer's protocol. After completion of the ELISA procedure standard curve was obtained using a Microsoft Excel spreadsheet. The concentrations of samples were obtained by feeding the readings in the same excel sheet after the generation of a standard curve.

RESULTS AND DISCUSSION

Enlargement of liver, kidney and inflammation of reproductive tract: Feed samples obtained during farm visit were checked for the presence of co-contamination of mycotoxins using ELISA kit. A total of 14 cases were

showing co-contamination of aflatoxin, ochratoxin and zearalenone in feed samples under present study. The percentage occurrence of co-contamination was found to be 28.5% (out of 49 feed samples tested). In this study, the mean level of aflatoxin, ochratoxin and zearalenone was 13.49693 ppb, 4.296286 ppb and 81.74543 ppb, respectively. The median value of aflatoxin levels observed was 6.3995 ppb, ochratoxin A was 2.9345 ppb and zearalenone was 83.703 ppb. Furthermore, these feed samples were also checked for the presence of cocontamination of other mycotoxins like trichothecene, DON, but were found to be negative. Kalita et al. (2021) reported presence of aflatoxin in feeds of Mizoram with the mean level of 11.254 ppb and the median level of 9.736 ppb for the first time. From the feed analysis, it can be concluded that case C/SEL/19/53,54 shows highest level of aflatoxin followed by case C/SEL/19/57 and C/ DUR/19/14. In case of ochratoxin highest level was observed in case C/DUR/18/09 followed by C/ICFAI/18/10 and C/SEL/19/53,54. While in case of zearalenone, highest level was observed in case C/ICFAI/18/10 followed by C/ SIP/19/16 and LALPUI/ICFAI/19/02. The relevant clinical signs and necropsy findings observed in these 14 positive birds/farms are depicted in Table 1. The observed necropsy changes in liver enlargement (hepatomegaly) observed in all cases, liver surface also revealed congestion (in all cases), yellow icteric discoloration (in 9 case) and fibrinous perihepatitis (in 7 case), in kidneys, congestion and remarkably congested observed in all cases, ascitic fluid deposition in 10 cases, cystic inflammation of reproductive tract in few cases (Fig. 1A-C). From the pathology point of view, it can be concluded that the main target organs under co-contamination of aflatoxin, ochratoxin and zearalenone are liver, kidneys and reproductive tract.



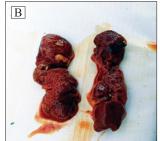
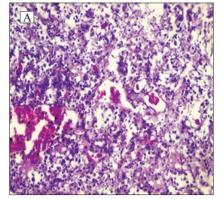


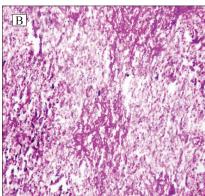


Fig. 1. A. Swollen congested enlarged liver; B. Congested and enlarged kidney appearing dark colored; C. Cystic Inflammation of the reproductive tract.

Table 1. Details of clinical signs and necropsy findings

Case name	Clinical observation	Necropsy findings
LALPUI/ ICFAI/19/02	Dull, depressed, ruffled feathers and showing huddling with stunted growth	Swollen congested liver, congested kidneys, congested bursa, flabby enlarged heart with congestion, ascitic fluid abdomen.
C/DUR/18/06	Stunted growth, poor body condition. Birds were dull, depressed and huddling.	Enlarged liver, pale cyanotic mucus membrane, congested kidneys, enlarged heart, oophoritis
C/DUR/18/07	Dullness, huddling, stunted growth with poor body condition.	Poor body condition, enlarged and yellowish liver, gall bladder distended with bile, congested kidneys, enlarged congested spleen.
C/LEI/18/08	Extremely weak birds with outstretched legs, cloacal swelling with staining, dullness, and depression.	Severely enlarged liver with marked congestion, enlarged congested spleen, congested, and enlarged kidney.
C/DUR/18/09	Birds were visibly sick with off feed, ruffled feather, huddling together and depressed.	Liver enlarged with congestion, kidney congested and swollen, mild enteritis, ascitic fluid abdomen, cystic bodied in the reproductive tract.
C/ICFAI/18/10	Ruffled feathers, depression, dullness, and most birds were off feed resulting into stunted growth, huddling together,	Poor body condition, liver tanned and presence of red congested areas, kidney congested and enlarged, bursa pale and flaccid, enteritis and congested swollen spleen, ascitic fluid abdomen, cystic bodies in the reproductive tract.
C/SIP/19/11	Birds noticeably dull depressed with ruffled feathers. Continuous death due to off feed, respiratory problems, open beak breathing.	Severe loss of breast muscle leading to prominent keel bone. Presence of breast blisters indicating cellulitis, pale musculature, liver with pinpoint hemorrhages, congested spleen, pale enlarged kidney.
C/SIP/19/12	Ruffled feathers, poor growth, dull, depressed birds, and huddling behavior.	Mildly congested liver with small foci of necrosis, moderately congested kidney, Pale and mild swollen bursa, congested spleen, enteritis.
C/DUR/19/14	Birds were dull, depressed with ruffled feathers. The body condition was poor and showing stunted growth.	Poor body condition, pale enlarged liver, enlarged and congested kidneys, ascitic fluid abdomen.
C/SIP/19/16	Lack of alertness, dull depressed with ruffled feather and showing huddling behavior.	Severely enlarged liver with marked congestion, enlarged congested spleen, congested and enlarged kidney, cystic bodies in the reproductive tract.
C/SIP/19/17	Extremely weak birds with outstretched legs, cloacal swelling with staining, dullness, and depression.	Swollen congested liver, congested kidneys, congested bursa, flabby enlarged heart with congestion, ascitic fluid abdomen, oophoritis.
C/SEL/19/51	Ruffled feathers, poor growth, dull, depressed birds, and huddling behavior.	Severely enlarged liver with marked congestion, enlarged congested spleen, congested, and enlarged kidney.
C/SEL/19/53,54	Ruffled feathers, depression, dullness, and most birds were off feed resulting into stunted growth.	Mildly congested liver with small foci of necrosis, moderately congested kidney, Pale and mild swollen bursa, congested spleen, enteritis, ascitic fluid abdomen, oophoritis.
C/DUR/19/57	Dullness, huddling, stunted growth with poor body condition.	Enlarged liver, pale cyanotic mucus membrane, congested kidneys, enlarged heart, oophoritis ascitic fluid abdomen.





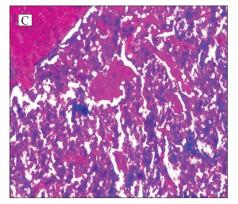
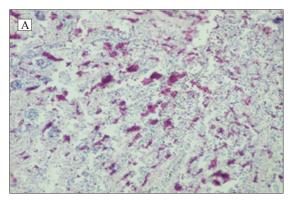


Fig. 2. A. Liver showing proliferation of fibrous structures in parenchyma with individualization of hepatocytes (H&E \times 400); B. Liver showing proliferation of bile canaliculi and brown staining hemosiderin (Luna- Ishak \times 100); C. Liver showing very few foci of collagen proliferation, however there were purple staining fibrinous structures present in the sinusoids (Masson's Trichrome \times 100.)

However, in aflatoxicosis, enlarged congested liver Khan *et al.* (2010), presence of necrotic foci on liver (Hashem and Mohamed 2009), swollen and congested kidneys (Khan *et al.* 2010) and congested spleen (Bedre *et al.* 2010) were reported earlier. Congested enlarged liver with necrotic foci and congested with enlarged kidneys followed by inflammation of reproductive tract and presence of fluid in the abdomen were reported for the first time in combined toxicity of aflatoxin, ochratoxin and zearalenone. Further findings included the observation of fibrinous perihepatitis in the liver, a disease not previously documented in the literature. Perusal of available literature did not reveal any other published article on pathology of combined aflatoxin, ochratoxin and zearalenone toxicity under natural condition.

Histopathological studies: The details histopathological lesions observed in liver were presence of necrotic foci in all cases, congestion of blood vessels and sinusoids and degenerating hepatocytes observed in all cases, fatty change in 10 cases. Non-uniform hepatocytes (in 8 cases), biliary stasis (in 5 cases), bile canaliculi proliferation observed in 7 cases diagnosed by Luna Ishak Staining. Loss of sinusoidal arrangement, cirrhotic changes observed in 5 cases diagnosed by Masson's trichrome staining (Fig. 2A-C). The details of histopathological lesions in kidneys were congestion of blood vessels observed in all cases, glomerular degeneration in 12 cases, tubular epithelial necrosis in 8 cases and cystic inflammation in in 8 cases (Fig. 3A-C).



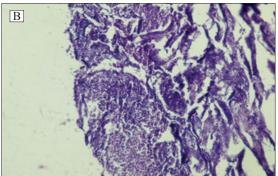


Fig. 3. A. Kidney showing extreme congestion of blood vessel and tubular necrosis (H&E \times 100); B. Ovary showing degenerative ova and inflammation (H&E \times 100).

In this combined toxicity of aflatoxin, ochratoxin and zearalenone, histological changes mainly observed in liver kidney and reproductive system. The common findings were congestion of blood vessels and sinusoids, fatty changes. Along with these findings, unique histopathological findings recorded were 5 case of cirrhotic changes (diagnosed by Masson's trichrome staining), 7 cases of bile canaliculi proliferation (diagnosed by Luna Ishak Staining). The case related to inflammation of reproductive system were additional finding obtained during this study. A review of the literature found no evidence of these histological abnormalities in chicken liver, kidney, and reproductive tract under natural condition of combined toxicity of aflatoxin, ochratoxin and zearalenone. Additionally, literature did not also reveal any other published article on histology of naturally occurring combined aflatoxin, ochratoxin and zearalenone toxicity in poultry.

The present study confirmed the presence of cocontamination of aflatoxin, ochratoxin and zearalenone in poultry feeds used in the Aizawl, Mizoram. The affected farms revealed mortality and pathological findings associated with aflatoxicosis, ochratoxicosis and zearalenone toxicity. The management practices on affected farms were faulty and contributed to the aggravation of pathology of mycotoxicosis in affected birds.

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REFERENCES

Anjum M A, Khan, S H, Sahota A W and Sardar R. 2012. Assessment of aflatoxin B1 in commercial poultry feed and feed ingredients. *Journal of Animal and Plant Science* 22: 268–72.

Annual Report (2022–2023). Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying Government of India, New Delhi, pp. 4.

Bancroft J D and Gamble D. 2008. *Theory and Practice of Histological Techniques*. 6th edn., Churchill Livingstone, UK, pp. 83–134.

Bedre D K, Kulkarni G B, Gangane G R, Mote C S and Dhaygude V S. 2010. Efficacy of 'Toxiroak' (herbal preparation) on gross and histopathological observations in mycotoxicosis in broilers. *Indian Journal of Veterinary Pathology* **34**(2): 141–44.

Hashem M A and Mohamed M H. 2009. Haemato-biochemical and pathological studies on aflatoxicosis and treatment in broiler chicks in Egypt. *Veterinaria Italiana* **45**(2): 323–37.

Kalita J J, Arya R S, Ravindran R, Singh Y D, Rajkhowa T K, Samanta A K, Choudhary O P, Subudhi P K, Suohu S, Kiran J and Behera B. 2021. A study on the pathology of spontaneous aflatoxicosis and local farming practices in broiler chicken farm in Aizawl, Mizoram, India. *Indian Journal of Animal Health* 60(2): 231–41.

- Khan S H, Shamsul H, Rozina S and Muhammad A A. 2011. Occurrence of aflatoxin B1 in Poultry feed and feed ingredients in Pakistan. *International Journal of Agro Veterinary and Medical Sciences* **5**: 30–42.
- Khan W A, Khan M Z, Khan A and Hussain I. 2010. Pathological effects of aflatoxin and their amelioration by vitamin E in white leghorn layers. *Pakistan Veterinary Journal* **30**(3): 155–62.
- Luna G L. 1968. Manual of Histologic staining methods. 3rd ed.
 McGraw-Hill Book Company, New York, USA, pp. 93–94.
 Park J H, Kim Y P, Kim I H and Ko S. (2014). Rapid detection of
- aflatoxin B1 by a bifunctional protein crosslinker-based surface plasmon resonance biosensor. *Food Control* **36**: 183–90.
- Parvathi D, Rajender R A and Krishna R V. (2017). Incidence of mycotoxins in poultry feeds and feed ingredients used in Warangal (TS), India. *International Journal of Life Science* **5**(3): 399–404.
- Reverberi M, Punelli F, Scarpari M, Camera E, Zjalic S and Ricelli A. 2010. Lipoperoxidation affects ochratoxin A biosynthesis in *Aspergillus ochraceus* and its interaction with wheat seeds. *Applied Microbiology and Biotechnology* 85: 1935–194.