A study on the pathology of spontaneous aflatoxicosis and local farming practices in broiler chicken farm in Aizawl, Mizoram, India

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Abstract

The study was conducted to investigate poultry feed contamination with aflatoxin, its effects on naturally exposed chicken and the role of local management practices in the overall scenario. During the investigation, different chicken farms in the Aizawl district of Mizoram state were surveyed and monitored for management practices, the occurrence of disease and mortality. The feed samples collected from affected farms were tested for the presence of total aflatoxin using commercially available competitive ELISA kits. The dead birds from affected farms were subjected to necropsy. Liver and kidneys from the dead birds of affected farms were processed for histopathological studies. Nine farms were found affected by aflatoxicosis. In this study, the mean levels of 11.254 ppb and the median level of 9.736 ppb of aflatoxin were found among the affected farms. Birds of the affected flocks revealed signs of illness like ruffled feathers, depression, dullness, huddling, poor growth, anorexia, respiratory distress and diarrhoea. Necropsy revealed pathological lesions in visceral and lymphoid organs. Histopathological analysis of liver and kidney revealed varying degrees of inflammatory, degenerative and necrotic lesions. It was also found that all affected farms were following poor management practices. A perusal of available literature did not reveal any studies on the presence of aflatoxin in poultry feed and mycotoxicosis in Aizawl, Mizoram.

Key words: Aflatoxin, Aizawl, Chicken, ELISA, Pathology

INTRODUCTION

Mizoram population is primarily non-vegetarian, and meats like pork and chicken are important parts of their diet. The poultry industry in the region is developing and faces numerous challenges. Low-grade mycotoxicosis leads to production losses and makes chicken susceptible to numerous other diseases, which increase mortality on a farm in the long run. *Aspergillus*, *Fusarium*, *Alternaria*, *Claviceps* and *Penicillium* genus of fungi are ubiquitous in nature and, under ideal conditions, often infect economically important crops

during storage, shipment and processing (Tamilmani *et al.*, 2020). Aflatoxins are produced by toxigenic strains of the fungi *Aspergillus flavus* and *A. parasiticus* in feed grains (Aneesh *et al.*, 2021). The toxicopathological range of aflatoxins is categorized as acute toxicity, chronic toxicity, carcinogenicity, teratogenicity and immunotoxicity (Huff *et al.*, 1984). Hepatocytes are the main site for bio-activation, and consequently liver is the major target site of toxicity. Kidneys are other important organs affected by aflatoxin. The gross changes seen in these organs occur

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due to degeneration, necrosis and inflammatory reaction at tissue level (Huff *et al.*, 1984; Dafalla *et al.*, 1987; Ledoux *et al.*, 1999). The toxins also cause pathological alteration in other body systems, such as lymphoid tissues (Dafalla *et al.*, 1987). Aflatoxins are encountered so frequently in feedstuffs that in recent times some innovations are being tried to reduce their activity in feed using diatomaceous earth (Lakkawar *et al.*, 2016), plants like *Aegle marmelos* and *Andrographis paniculata* (Aneesh *et al.*, 2021) and lactic acid bacteria fermentation (Seyedjafarri, 2021). The occurrence of aflatoxicosis in poultry birds had been reported for a very long time in India. Aflatoxicosis was first reported in India in the year 1966 from the Mysore district of Karnataka state, where 2219 chicks died in one week (Reddy and Raghavender, 2007). Acharya *et al.* (1984) found aflatoxin in rice samples from Odisha. In the year 1986, an episode of aflatoxicosis had occurred in ducklings due to ingestion of contaminated feed in Tripura (Roy *et al.*, 1989). In Assam, Bihar and Tripura, the median value of 15 µg/ kg of aflatoxin B1 were reported in parboiled rice (Subhash *et al.*, 2012). Recently, Parvathi *et al.* (2017) surveyed and reported contamination of various poultry ration and feed ingredients with mycotoxins, including aflatoxin, in Warangal, Andhra Pradesh. A perusal of available literature did not reveal any studies on the presence of aflatoxin in poultry feed and its effects on chicken in Aizawl, Mizoram. Consequently, the study was designed to investigate poultry feed contamination with aflatoxin, its effects on naturally exposed chicken and the role of local management practices in the overall scenario.

MATERIALS AND METHODS

Sampling: A total of 73 chicken farms were investigated from May 2018 to May 2019 during the study period. The farms were regularly visited and monitored for episodes of disease and mortality during the period. The ongoing management practices on the farm were studied and recorded whenever any farm revealed noticeable mortality, as shown in Table 1.

Feed samples from the affected farms were collected for ELISA based estimation of aflatoxin.

Pathology: 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 lesion 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 of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl. The standard procedure of necropsy examination followed included external examination followed by an internal examination of the carcass. The internal examination included an investigation of all the visceral organs for pathological lesions. All changes and lesions were carefully recorded and photographed.

Representative tissue samples from the parts of the liver and kidney which were 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). The observations were recorded and photographs were taken whenever necessary.

ELISA-based estimation of aflatoxin: Total aflatoxin in feed samples was estimated using a commercially available competitive ELISA kit (Tecna Srl, Italy; Catalogue code- MA210/ MA211). First, the methanolic extract was prepared from finely ground samples as recommended in the kit. The grinding of samples was performed with the help of an automated feed grinder at the department of Animal Nutrition of the institute. The ELISA procedure was performed at the Department of Physiology and Biochemistry of the institute. It was

conducted according to the manufacturer's protocol in a plastic microwell plate that had been coated with anti-aflatoxins antibody. The mixing of reagents was done in the premixing wells provided in the kit. The enzyme labelled aflatoxin, the standard solutions, and samples were mixed in it using micro-pipettes fitted with disposable tips. Then the mixtures were transferred into the respective wells of the anti-aflatoxin-microtiter plate. Incubation was allowed so that free aflatoxins in the standard solutions or test samples could compete with enzyme-labelled aflatoxin for the anti-aflatoxins antibody binding sites on the solid phase. After the incubation, a wash was performed to remove any unbound enzyme conjugate and aflatoxin molecule. Then, the bound enzyme activity was determined by adding a fixed amount of a proprietary chromogenic substrate provided with the kit. The enzyme converted the colourless chromogen into a blue product. The addition of the stop reagent led to a colour change from blue to yellow. Now the absorbance was immediately measured with a microplate reader at 450 nm. Standard curve from the readings of standard aflatoxin solutions was obtained using a Microsoft Excel spreadsheet downloaded from the company website www.tecnalab.com. The concentrations of samples were obtained by feeding the readings in the same excel sheet after the generation of a standard curve.

RESULTS

Management practices on affected farms:

The details of the results of the investigation on management practices on studied farms are given in Table 2. All farms fed commercially available broiler mash purchased from local vendors. None of the farms performed vaccination in the reared birds. However, the chicks had received vaccines only at the hatchery (IB, MD and ND) as per the available information. Only two farms were having concrete structures (C/DINGPUI/19/37 & C/SIP/19/15), and the rest others were constructed with bamboo and wood. Among the studied farms, only one farm (C/SIP/19/15) was using municipal water supply apart from rainwater and

hill stream harvested water. Rest all farms used stored hill stream water in the dry season, and while monsoons the rainwater harvested through the roof side harvesting system. All farms except two (C/SIP/19/15; C/DINGPUI/19/37), which were of concrete construction, had very poor ventilation and no provision for sunlight entry in the shed. The use of bamboo and wood was the most common construction material. Three farms had revealed remarkable odour of ammonia, and one had a strong putrid odour. Most farms had slated bamboo floors, but still, an ammonia smell was observed. Farm C/LUNG/19/13 had pig and poultry shed very close to each other, which is not good with regards to prevention of genetic shift and drift of influenza virus. In farm C/DINGPUI/19/37 the litter material was changed every 2-3 days. The farmer performed it to prevent diseases. The practice is not good for farm economics. Most farms had a problem of poor ventilation, biosecurity and overall poor hygienic practices.

ELISA: Feed samples from a total of 9 farms (12.32%) tested positive for the presence of total aflatoxin. The results of ELISA estimation of aflatoxin positive samples are given in Table 1. In the present study, the mean of aflatoxin levels observed was 11.254 ppb, and the median was 9.736 ppb. The mortality estimate of studied farms was variable, with a minimum of 3.22% (C/LUNG/18/29) to a maximum of 42.85% (C/SIP/18/18). The most affected age group was 20-30 days with 5 farms (55.55%) (C/DUR/18/19, C/SIP/18/18, C/DINGPUI/19/37, C/SEL/19/38, 40 and C/SIP/19/15) out of 9 falls in the range.

Clinical signs: The details of clinical signs observed on studied farms are given in Table 3. All affected flocks (100%) revealed signs of illness like ruffled feathers, depression and dullness to varying degrees. Huddling of chicks was noticed in three (33.33%) farms (C/SIP/18/18, C/LUNG/19/29 and C/LUNG/19/36). Four (44.44%) farms revealed remarkably poor growth according to the age of birds (C/SIP/18/18, C/DUR/18/19, C/SEL/19/33 and C/LUNG/19/36). However, anorexia was

noticeable in only two (22.22%) farms (C/LUNG/19/13 and C/LUNG/19/29). Two (22.22%) farms revealed respiratory problems and dyspnea (C/SIP/19/15 and C/SEL/19/38, 40). Diarrhoea was seen in one (11.11%) farm (C/SIP/19/15), while another one (11.11%) farm revealed remarkable pasting of vent among the affected birds (C/DINGPUI/19/37).

Necropsy finding: The details of necropsy findings in dead birds from studied farms are given in Table 3. All nine farms (100%) revealed hepatic lesions with necrosis (Fig. 1) and extremely friable liver easily rupturing (Fig. 2) in one farm (C/DUR/18/19), the pale liver in two farms (C/SIP/18/18 and C/LUNG/19/13) and congestion in five farms (C/LUNG/19/29, C/SIP/19/15, C/SEL/19/33, C/LUNG/19/36, C/DINGPUI/19/37 and C/SEL/19/38, 40). All nine farms (100%) revealed kidney lesions, with two farms showing pale kidneys (Fig. 3) with urate retained (C/SIP/18/18 and C/DUR/18/19) and the remaining all revealed congestion. Five farms (55.55%) revealed enteritis in dead birds (C/LUNG/19/29, C/SEL/19/33, C/LUNG/19/36, C/DINGPUI/19/37 and C/SEL/19/38, 40). Emaciation was observed in five (55.55%) farms (C/SIP/18/18, C/DUR/18/19, C/LUNG/19/29, C/LUNG/19/13 and C/SIP/19/15). In five farms (55.55%), varying degrees of pathological lesions were observed in the spleen like congestion (C/SIP/18/18&C/SEL/19/38, 40), paleness with flaccid structure (C/DUR/18/19) and congestion with swelling (C/SEL/19/33& C/LUNG/19/36). Three farms (33.33%) revealed gross lesions in the thymus, with one farm having birds with swollen and haemorrhagic thymus (C/LUNG/19/29) and two with congestion (C/SIP/19/15 and C/SEL/19/38, 40). Three farms (33.33%) revealed gross pathological changes in bursa, with bursal swelling in birds of two farms (C/LUNG/19/29 and C/SEL/19/38, 40) and pale flaccid bursa in one farm (C/LUNG/19/36). Two farms (22.22%) revealed ascites and hydropericardium (C/SEL/19/33 and C/DINGPUI/19/37), while one farm (11.11%) had only hydropericardium (Fig. 1) (C/SIP/19/15).

Histopathology of liver: The details of histopathological lesions in livers of studied cases are given in Table 4. Blood vessel congestion in the liver was shown by all 9 cases (100%) while sinusoidal congestion was shown by 8 cases (88.89%) except (C/SEL/19/38,40). All cases revealed variable degenerative changes in hepatocytes where fatty change was shown by 5 cases (55.55%) (C/LUNG/19/29, C/LUNG/19/13, C/SIP/19/15, C/LUNG/19/36 and C/DINGPUI/19/37), cellular swelling by 1 case (11.11%) (C/SEL/19/33), necrosis in 2 cases (Fig. 4) (22.22%) (C/DINGPUI/19/37 and C/SEL/19/38, 40) and individualization of hepatocytes (Fig. 4 and 5) in 1 case (11.11%) (C/SIP/18/18).

Histopathology of kidneys: The details of histopathological lesions in kidneys of studied cases are given in Table 4. Tubular epithelial necrosis (Fig. 6) was shown by all 9 cases (100%), while blood vessel congestion was shown by 8 cases (Fig. 6) (88.89%) except (C/SEL/19/38,40). Degenerative changes in glomeruli in was seen in 5 cases (Fig. 6) (55.55%) (C/SIP/18/18, C/SEL/19/33, C/LUNG/19/36, C/DINGPUI/19/37 and C/SEL/19/38, 40).

DISCUSSION

The present investigation was conducted in rural parts of Aizawl district of Mizoram. The farms targeted were small units with flock sizes varying from as low as 14 birds to 225 birds (Table 1). Most of the farms were maintained in the residence of the farmers. They rear commercial broiler chicks (Cobb strains) available from hatcheries from the neighbouring state of Assam. A peculiar practice in the region is that birds are reared for many months, frequently even more than six months. Very few farmers performed vaccination of birds, and in the present study, none of the flocks was vaccinated on-farm and had only received hatchery vaccination. The birds were fed commercially available formulated poultry ration sold by local vendors. The farmers did not keep systematic records and managed the farms according to rough estimates or by any remarkable events of

Table 1. Results of ELISA with sample details and mortality

Sl No	Case No	Aflatoxin level (ppb)	Date	Breed/strain	Age	Approximate duration of feeding suspected feed	Number of dead birds) (mortality %	Flock size	Type of farm/unit
1	C/SIP/18/18	13.326	20-09-18	Cobb	21 days	Since the beginning	6 (42.85%)	14	Unorganized
2	C/DUR/18/19	6.039	15-10-18	Cobb	21 days	Since the beginning	8 (13.33%)	60	Unorganized
3	C/LUNG/19/29	7.522	12-01-19	Cobb	Mixed-age group of 20 days, 35 days and 56 days	Since the beginning	4 (3.22%)	124	Unorganized
4	C/LUNG/19/13	20.829	16-01-19	Cobb	67 days	Since the beginning	5 (16.67%)	30	Unorganized
5	C/SIP/19/15	14.727	22-1-19	Cobb	23 days	Since the beginning	11 (42.30%)	26	Organized
6	C/SEL/19/33	10.096	12-03-19	Cobb	40 days	Since the beginning	5 (12.50%)	40	Unorganized
7	C/LUNG/19/36	9.275	14-03-19	Cobb	45 days	Since the beginning	4 (6.78%)	59	Unorganized
8	C/DINGPUI/19/37	9.736	19-03-19	Cobb	30 days	Since the beginning	5 (16.67%)	30	Organized
9	C/SEL/19/38,40	9.736	20-03-19	Cobb	30 days	Since the beginning	25 (11.11%)	225	Unorganized

Table 2. Details of farm management practices

Sl No	Case No	Feed source	Water source	Farm construction, hygiene and water source	Vaccination status
1	C/SIP/18/18	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Bamboo poultry shed in backyard of house, poor ventilation, no sunlight entry, feeder and waterer used were home utensils	No vaccination by farmers
2	C/DUR/18/19	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Bamboo poultry shed, poor ventilation, no heat source or brooder, overcrowding by farmer	No vaccination by farmer
3	C/LUNG/18/29	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Bamboo and wood farm, slatted bamboo floor, mild ammonia smell, overcrowding by farmers	No vaccination by farmers
4	C/LUNG/19/13	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Bamboo and wood farm, hygiene not properly maintained, poultry and pig shed located very close, foul putrid smell in farm complex,	No vaccination by farmers
5	C/SIP/19/15	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting and municipal supply	Concrete shed, litter was wet	No vaccination by farmers
6	C/SEL/19/33	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting.	Bamboo shed, good hygienic farm but there was lack of ventilation and sunlight, overcrowding, water in use was stored, feed was stored in gunny bags along with new litter material bags in the shed itself.	No vaccination by farmers
7	C/LUNG/19/36	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Bamboo shed, poor ventilation and sunlight, poor hygiene, dirty feeder and waterer, remarkable ammonia smell.	No vaccination by farmers
8	C/DINGPUI/19/37	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Concrete shed located at hilltop, good spacing for birds, overall hygiene was good but no foot bath at entrance, farmer practices litter replacement in every 2-3 days.	No vaccination by farmers
9	C/SEL/19/38,40	Commercial broiler ration purchased from local vendor	Rainwater and stream harvesting	Bamboo shed, poor ventilation and sunlight with only one side opened, wet litter, overcrowding, severe ammonia smell,	No vaccination by farmers

Table 3. Details of clinical signs and necropsy findings

Sl No.	Case No	Clinical observations	Necropsy findings
1	C/SIP/18/18	Ruffled feathers, poor growth, dull, depressed birds and huddling behaviour.	Prominent keel bone indicating loss of breast muscle, breast blisters with cellulitis in subcutaneous tissue, pale musculature, liver pale with pinpoint haemorrhages, congested spleen, pale kidney with white striations indicating urate retention in tubules, hock joint swollen and revealed oedema on cutting
2	C/DUR/18/19	Dull depressed birds with ruffled feathers and remarkably stunted growth.	Prominent keel bone indicating loss of breast muscle, pasting of vent, rib impression and necrotic foci on liver, there was rupture of liver with haemorrhage in one of the birds, dark bile, juvenile ovaries appeared hyperaemic, haemorrhagic proctitis in one of the bird, kidney swollen with white striations indicating urate retention in tubules, spleen pale and flaccid
3	C/LUNG/19/29	Birds were visibly sick, off feed, showing huddling, ruffled feathers and depression.	Prominent keel bone indicating loss of breast muscle, congested liver and kidney, swollen haemorrhagic thymus, swollen bursa and haemorrhagic enteritis
4	C/LUNG/19/13	Ruffled feathers, depression, dullness and most birds were off feed.	Poor body condition, pale breast muscle, yellow liver, pale cyanotic comb, periorbital and palpebral edema, congested kidneys, juvenile ovaries appeared congested, flabby enlarged heart
5	C/SIP/19/15	Respiratory distress with open mouth breathing and diarrhoea	Keel bone prominent with emaciated breast muscle, liver congested, kidney congested, mildly congested thymus, presence of mild hydropericardium (2 mL fluid)
6	C/SEL/19/33	Dullness, depression, poor body condition and poor growth.	Poor body condition and musculature, swollen congested liver, congested kidney, spleen with severe congestion and mild swelling, haemorrhagic enteritis, ascites, hydropericardium with rounded flaccid heart, extreme congestion in lungs
7	C/LUNG/19/36	Ruffled feathers, huddling and poor growth.	Poor body condition and musculature, pale carcass, liver tanned and presence of red congested areas, kidney congested, trachea with mild mucous, bursa pale and flaccid, enteritis and congested swollen spleen
8	C/DINGPUI/19/37	Extreme weakness indicated by outstretched legs, cloacal straining indicated by swollen cloacal opening, dullness and depression.	Poor body condition, liver enlarged and tanned with congestion, ascites, hydropericardium with rounded flaccid heart, extremely congested and pneumonic lungs, kidney congested and swollen, mild enteritis
9	C/SEL/19/38,40	Continuous death with ascites and respiratory problems, open beak breathing, birds noticeably dull depressed, and ruffled feathers	38- Moderate size of pectoral muscles, little fat in abdominal coelom, liver congested with small necrotic foci, kidney congested, congested spleen and mild swelling of bursa40- Liver swollen and enlarged, kidney swollen and congested, spleen was tanned, thymus enlarged and congested, enteritis with ballooning, right ventricle of heart enlarged and flaccid, severe congestion of lungs

Table 4. Histopathological lesions in liver and kidneys of studied cases

Sl No	Case No	Liver	Kidney
1	C/SIP/18/18	Blood vessel congestion, necrotic areas with individualization of the hepatocytes, sinusoidal congestion	Blood vessel congestion, tubular and glomerular degeneration
2	C/DUR/18/19	Blood vessel congestion, necrotic foci, sinusoidal congestion	Extensive blood vessel congestion, tubular necrosis
3	C/LUNG/19/29	Blood vessel congestion, sinusoidal congestion, focal areas of fatty change	Extreme blood vessel congestion, and tubular necrosis
4	C/LUNG/19/13	Fatty change, blood vessel congestion, sinusoidal congestion	Blood vessel congestion and tubular necrosis with epithelial exfoliation
5	C/SIP/19/15	Fatty change, blood vessel congestion, sinusoidal congestion	Blood vessel congestion and tubular necrosis with exfoliation of epithelium in lumen of tubules
6	C/SEL/19/33	Blood vessel congestion, sinusoidal congestion, pale cytoplasm indicating cellular swelling	Extensive tubular necrosis, vacuolation in the glomerulus, necrotic tubular epithelium
7	C/LUNG/19/36	Blood vessel congestion, sinusoidal congestion, fatty change	Blood vessel congestion, tubular necrosis, degeneration of glomerulus
8	C/DINGPUI/19/37	Blood vessel congestion, sinusoidal congestion, focal areas of fatty change and necrosis with infiltration of MNC, micro thrombosis	Extensive blood vessel congestion, tubular necrosis, glomeruli with vacuolation and micro thrombosis
9	C/SEL/19/38,40	Distorted sinusoidal arrangement, areas of necrosis, especially around the blood vessels	Extensive tubular necrosis, glomerulus with degenerative changes and swollen tuft of capillaries

Fig. 1. Liver of dead chicken showing pale, swollen and necrotic liver (Red Arrow).
Heart is enlarged with cloudy pericardium and moderate level of hydropericardium

Fig. 2. Liver of dead chicken showing swelling and pale necrotic patches (yellow arrow).
The liver was friable indicated by breakages in the structure (blue arrow)

Fig. 3. Kidneys of dead chicken showing swelling, paleness and striated appearance (yellow arrow)

Fig. 4. Liver, chicken: Liver shows numerous necrotic foci in parenchyma (arrow). The affected area shows loss hepatocytes, detached cells from surrounding tissue with eosinophilia (H&E, X 100)

Fig. 5. Liver, chicken: Liver shows numerous necrotic, individualized hepatocytes (arrow). The necrotic hepatocytes have pyknotic nuclei, eosinophilic cytoplasm and there is total loss of normal hepatic cord arrangement around sinusoids (H&E, X 600)

Fig. 6. Kidney, chicken: Kidney shows tubules with necrotic epithelium showing loss of cellular integrity, pyknotic nuclei and exfoliation in tubular lumen (red arrow). There is extensive capillary congestion (yellow arrow). Glomeruli show degenerative changes with discontinuous Bowman's capsule showing adhesions with capillary tuft surface which itself shows non-uniform cellularity and prominent mesangium (Green arrow) (H&E, X 100)

mortality. Low-grade sporadic mortality is generally neglected by farmers. Changes in feed and water consumption were generally not noticed by farmers, sometimes even during outbreaks. Only those farms were investigated for the presence of mycotoxins in feed where farmers reported mortality and flock were clinically sick.

Feed samples from a total of 9 farms were found positive for the presence of aflatoxin. In the present study, the mean of aflatoxin levels observed was 11.254 ppb, and the median was 9.736 ppb. Guerre (2016) reviewed that the maximum permissible level of aflatoxin B1 for mature poultry birds in complete feed is 20 ppb. For immature birds, this level is even lower. All birds in the presented study were immature broiler chicken. Dutta and Das (2001) reported AFB1 contamination in animal feed samples found positive for the presence of aflatoxigenic fungi in north India at an average level of 412 ± 154 ppb as estimated by ELISA and TLC. This is very high as compared to the present study since those feed samples were found positive for fungal growth. Thirumala-Devi *et al.* (2002) reported that 38% of studied poultry feed and ingredients from different places in India had aflatoxin contamination at an average level of 10 µg/kg (equivalent to 10 ppb) as estimated by Indirect Competitive ELISA, which is comparable to our findings. Another comparable report was from Vijayasamundeeswari *et al.* (2009), where they found the prevalence of aflatoxin B1 contamination in 30 out of 53 poultry feed samples from Tamil Nadu at levels ranging from 0.7 to 31.6 ppb as estimated by ELISA. Further, Kannan *et al.*, (2014) found that aflatoxin B1 contamination of 51 poultry feed samples in Tamil Nadu, were at levels ranging from 0 to 160.7 ppb as estimated by HPLC. They also found that the levels of AFB1 in 29% of the samples exceeded 20 ppb.

None of the farms performed vaccination on their chicks. The chicks had received vaccines only at the hatchery. This makes birds susceptible to infectious diseases. Only two farms had proper concrete structures, and the

rest others were constructed with temporary bamboo and wood structures which do not provide for proper housing requirements for the broiler birds. Bamboo farms had very poor ventilation, which increases the susceptibility of birds to various diseases, including ascites syndrome. The accumulation of ammonia and other gases from the putrefaction of organic matter and excreta on-farm further elevates the stress on birds. All farms used rainwater and hill stream harvested water at some of the other points of the year. A farm had multiple age groups on the same shed, which makes younger birds easily exposed to pathogens harboured by older age group birds. Another farm had pig and poultry shed very close to each other, which is a disastrous practice with regards to prevention of genetic shift and drift of avian and porcine influenza virus. Most farms had problems of poor ventilation, biosecurity and overall poor hygienic practices. It can be concluded that most farms were grossly mismanaged, which made birds susceptible to diseases.

Gholami-Ahangaran *et al.* (2016) reported severe congestion, degeneration, and necrosis in the liver and kidney in chickens that received aflatoxin. Mughal *et al.* (2017) reported hydropic degeneration, fatty vacuolar degeneration and proliferation of bile duct in hepatocytes of chicken intoxicated with aflatoxin B1. They also found an imbalance between the reactive oxygen species (ROS) and antioxidant defense systems. Further they also reported that there is increased apoptosis as apoptotic receptors (FAS, TNFR1) are upregulated and antiapoptotic proteins (XIAP, BCL-2) are downregulated. Slizewska *et al.* (2019) reported vacuolar degeneration, biliary hyperplasia and nodular cirrhosis in the liver of broiler chickens fed with aflatoxin B1. Kidneys revealed enlargement of glomeruli in affected chicken. They found that probiotic supplementation in the diet reduces the changes in liver and kidneys of aflatoxin B1 fed birds. Ates and Ortatatli (2021) reported histopathological lesions of hydropic degenerations, fatty change, periportal

infiltration of inflammatory cells, hepatocytes arranged in acinar pattern and proliferation of biliary ducts in broiler chicken. They also found that there was a significant increase in nuclear receptors of AhR, PXR and CAR. Further, there was also increased enzyme activity of CYP1A1 and CYP2A6. These are responsible for the bioactivation of aflatoxin in the liver. They reported the protective effects of *Nigella sativa* seeds and its active ingredient thymoquinone on the liver. All affected flocks revealed some of the other signs of illness with the varying presence of anorexia, diarrhoea, respiratory distress and weight loss. It was clear that the birds were clinically sick. Similar findings had been earlier reported by other workers (Dafalla *et al.*, 1987; Fink-Gremmels, 1998; Bedre *et al.*, 2010; Khan *et al.*, 2010). The necropsy findings seen in the present study were also reported by other workers like enlarged congested liver (Dafalla *et al.*, 1987; Bedre *et al.*, 2010; Khan *et al.*, 2010), presence of necrotic foci on the liver (Dafalla *et al.*, 1987; Hashem and Mohamed, 2009; Bedre *et al.*, 2010), swollen and congested kidneys (Dafalla *et al.*, 1987; Fernandez *et al.*, 1995; Khan *et al.*, 2010) and congested spleen (Dafalla *et al.*, 1987; Bedre *et al.*, 2010). The common histopathological changes observed in the liver-like presence of blood vessel congestion and sinusoidal congestion (Bedre *et al.*, 2010; Khan *et al.*, 2010; Gholami-Ahangaran *et al.*, 2016;

Mughal *et al.*, 2017; Slizewska *et al.*, 2019; Ates and Ortatatli, 2021), individualization of hepatocytes and vacuolated cytoplasm had been reported by other workers (Singh *et al.*, 1994; Bedre *et al.*, 2010). In kidneys, the changes like extreme blood vessel congestion, glomerular hypercellularity and mesangium thickening and tubular necrosis had been found earlier (Bedre *et al.*, 2010; Khan *et al.*, 2010; Slizewska *et al.*, 2019). The histopathological lesions in the liver and kidneys are consistent with aflatoxicosis lesions. This indicates that, at the observed levels of concentration, aflatoxin can exert toxic effects on the liver and kidneys of broiler chicks.

The presented study confirms the presence of aflatoxin in poultry feeds used in the Aizawl region. The affected farms revealed mortality and pathological findings associated with aflatoxicosis. The management practices on affected farms were faulty and contributed to the aggravation of pathology of mycotoxicosis in affected birds.

Conflict of interest: Authors have no conflict of interest in this study.

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