RESEARCH ARTICLE

Pathology of Co-contamination of Mycotoxins (Aflatoxin and Ochratoxin) in Poultry Farms in Aizawl, Mizoram, India

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Abstract

The study was conducted to investigate the pathology of naturally occurring co-contamination of mycotoxins in poultry farms and the role of local management practices in the overall scenario. During the investigation, different chicken farms (n=73) in the Aizawl district of Mizoram state (India) 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 Aflatoxin and Ochratoxin by ELISA kits. The dead birds were subjected to necropsy. Liver and kidneys from the dead birds were processed for histopathological studies. Ten feed samples out of 49 tested (20.4%) were found affected by co-contamination with Aflatoxin and Ochratoxin. In this study, the mean level of Aflatoxin and Ochratoxin was 13.302 ppb and1.822 ppb, and the median level 12.143 ppb and1.674, ppb, respectively. Clinical signs were ruffled feathers, depression, dullness, huddling, poor growth and , anorexia. Necropsy revealed pathological lesions in visceral and lymphoid organs. Histopathological findings were inflammations, degenerative, and necrotic lesions in liver and kidneys. Poor management practices were observed in affected farms. 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, Mizoram.

Key words: Aflatoxin, Aizawl, ELISA, Ochratoxin, Pathology, Poultry.

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Introduction

Chicken is the predominant domesticated poultry bird in India, and while poultry farming was traditionally a backyard activity until 1960, 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. 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, and mycotoxicosis influence the poultry sector.

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 mycotoxicosis. (Anjum *et al.,* 2011). *Aspergillus flavus, Aspergillus parasiticus, and Penicillium puberulum* produce the six major aflatoxins: B1, B2, G1, G2, M1, and M2. AFB1 (Aflatoxin B1) is the most toxic among them, primarily affecting the liver and classified as a Group I carcinogen by the International Agency for Research on Cancer (Anonymous, 2002). Ochratoxins are produced by *Aspergillus* and *Penicillium*, these include certain members of *Aspergillus ochraceus* group and *Penicillium verrucosum* type I and II (Reverberi *et al.,* 2010). Among 3 types of Ochratoxin A, B and C, ochratoxin A (OTA) is the most harmful one. Ochratoxins are considered as powerful nephrotoxins, carcinogens, teratogens, and immune toxins (Ceci *et al.,* 2008). Parvathi *et al.* (2017) and Kalita *et al.* (2021) reported contamination of various poultry ration and feed ingredients with mycotoxins, including aflatoxin, in Warangal, Andhra Pradesh, and Aizawl, Mizoram. Ochratoxicosis occurrences, often co-contaminant with other mycotoxins like aflatoxin, have been reported

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worldwide. Aflatoxicosis symptoms include inappetence, reduced growth, feather picking, ataxia, convulsions, and opisthotonos leading to death. Ochratoxin-contaminated feed may cause renal diseases, air sacculitis, reduced feed intake, and poor growth. Despite these known risks, there is a literature gap on the combined toxicity of aflatoxin and ochratoxin in poultry feeds, especially in the Northeastern Region of India. This study investigates co-contamination in poultry feeds, its pathology, and evaluates the impact of local management practices in Aizawl, Mizoram.

Meterials and Methods

Collection and Processing of Sample

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 birds to 80 birds. Since there was no slaughter of birds involved, approval of Institutional animal ethics committee was not required/sought. 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 based estimation of various mycotoxins.

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 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 the 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).

Mycotoxin Estimation

Total Aflatoxin and Ochratoxin in feed samples was estimated using a commercially available competitive ELISA kits (TecnaSrl, Italy; Catalogue code- MA210/ MA211 &OR360/ OR361). 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 (www.tecnalab.com) (Fig 1a,b). The concentrations of samples were obtained by feeding the readings in the same excel sheet after the generation of a standard curve.

Special Staining

Procedures of Special Staining (Luna Ishak staining, Masson's Trichrome stain, Modified Brown and Brenn staining) done were as follows.

The Luna Ishak staining method was used to demonstrate bile canaliculi in the liver samples, as outlined by Luna in 1968. Masson's Trichrome staining was used for the demonstration of collagen fibers (Luna, 1968). The standard protocol with a modification, methylene blue instead of Aniline blue was followed. The Modified Brown and Brenn staining methods were applied to visualize bacteria in tissue samples, following Luna's protocol (Luna ,1968). Air dried slides were then mount with paraffin. The stained sections/slides with all three methods were then observed under the light microscope and the histopathological lesions were recorded.

Results and Discussion

Feed samples obtained during farm visit were checked for the presence of co-contamination of mycotoxins using ELISA kits.

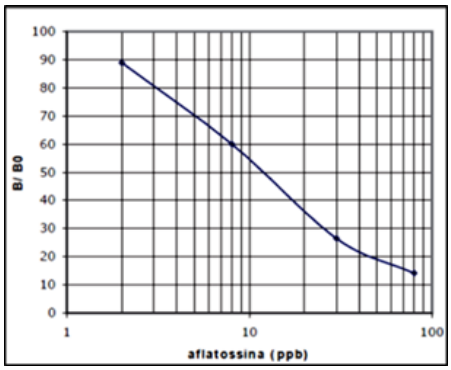

Fig. 1a: Standard curve for Aflatoxin ELISA kit

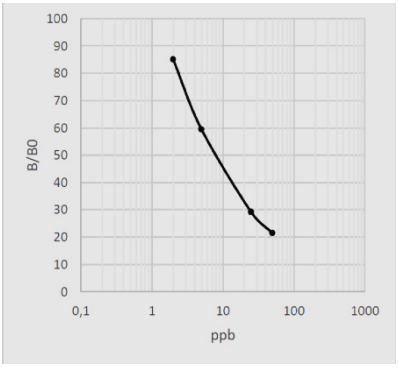

Fig. 1b: Standard curve for Ochratoxin ELISA kit

A total 10 cases showed co-contamination of aflatoxin and ochratoxin in feed samples under this study. The percentage occurrence of co-contamination was thus found to be 20.4% (10 out of 49 feed samples tested positive). In the present study, average of Aflatoxin level observed was 13.302 ppb and Ochratoxin A was 1.822 ppb and median of Aflatoxin level was 12.143 ppb and Ochratoxin A was 1.674 ppb. Furthermore, these feed samples were also checked for the presence of co-contamination of other Mycotoxins like Trichothecene, DON, Zearalenone, but were found to be negative. Similar findings were also reported by Thirumala-Devi *et al.* (2002). Additionally, Kalita *et al.* (2021) reported presence of Aflatoxin in feeds of Mizoram state 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 /18/20 shows highest level of aflatoxin followed by case C/ SEL/ 18/23 and C/DINGA/19/35, while in case of Ochratoxin highest level was observed in case C/SEL/18/19 followed by C/SEL/18/21 and C/SEL/18/23. The relevant clinical signs and necropsy findings observed in these 10 positive birds/farms are depicted in Table 1.

The common clinical signs observed by affected flocks were ruffled feathers, depression, dullness, stunted growth due to off feed. Some birds were extremely weak with outstretched legs, cloacal swelling with staining, followed by respiratory problems, open beak breathing and huddling behaviour.

The observed necropsy changes in targeted organs can be described as presence of rib impression on liver surface (in 7 cases), mild hepatomegali observed in 3 cases (Fig. 2), liver surface also revealed congestion (in 10 cases), necrotic foci in 5 cases (Fig. 3), yellow icteric discoloration (in 2 case) and fibrinous perihepatitis (in 1 case), kidneys in 10 cases were found to be remarkably congested and in 5 cases were also swollen (Fig 4,5).

The details of histopathological lesions observed in liver were presence of necrotic foci in 5 cases (Fig. 6), congestion of blood vessels and sinusoids and degenerating hepatocytes observed in 10 cases (Fig.7), fatty change in 4 cases. Non-uniform hepatocytes in 3 cases, biliary stasis in 2 cases, bile canaliculii proliferation observed in 2 cases diagnosed by Luna Ishak Staining (Fig.8). Loss of sinusoidal arrangement, cirrhotic changes observed in 1 case diagnosed by Masson's trichrome staining (Fig.9), Coagulative necrotic

Table 1: Details of clinical signs and necropsy findings

Sl. No.	Case name	Clinical observation	Necropsy findings
1	C/ SEL /18/02,03,04	Dull, depressed, ruffled feathers and showing huddling with stunted growth	Swollen congested liver, congested kidneys, congested bursa, flabby enlarged heart with congestion.
2	C/SIP/18/05	Stunted growth, poor body condition. Birds were dull, depressed and huddling.	Enlarged liver, pale cyanotic mucus membrane, congested kidneys, enlarged heart, oophorities
3	C/ SEL /18/18	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.
4	C/ SEL /18/19	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.
5	C/ SEL /18/20	Birds were visibly sick with off feed, ruffled feather, huddling together and depressed.	Poor body condition, liver tanned and presence of red congested areas, kidney congested and enlarged, bursa pale and flaccid, enteritis and congested swollen spleen.
6	C/ SEL /18/21	Ruffled feathers, depression, dullness, and most birds were offfeed resulting into stunted growth.	Liver enlarged with congestion, kidney congested and swollen, mild enteritis.
7	C/SIP/18/23	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 pin point haemorrhages, congested spleen, pale enlarged kidney
8	C/ SEL /19/26	Ruffled feathers, poor growth, dull, depressed birds, and huddling behaviour	Mildly congested liver with small foci of necrosis, moderately congested kidney, Pale and mild swollen bursa, congested spleen, enteritis
9	C/DINGA/19/35	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.
10	C/SEL/19/38,40	Lack of alertness, dull depressed with ruffled feather and showing huddling behaviour	Severely enlarged liver with marked congestion, enlarged congested spleen, congested and enlarged kidney.

in liver revealed presence of colony of Gram-positive bacteria observed in one case which was later diagnosed by modified Brown and Brenn staining (Fig. 10 ,11). The details of histopathological lesions in kidneys were congestion of blood vessels observed in 10 cases (Fig.12), glomerular degeneration in 7 cases (Fig.13), tubular epithelial necrosis in 5 cases (Fig.14) and microthrombosis was observed in 1 case (Fig.15).

From the pathology point of view, it can be concluded that the main target organs under mycotoxicosis are liver and kidneys. Enlarged liver and kidneys with presence of necrotic foci, followed by liver and kidney congestion were observed under this co-contamination of Aflatoxin and Ochratoxin. 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 enlarged kidneys with congestion were reported for the first time in combined toxicity of mycotoxin. Additionally, we observed gross findings such as fibrinous perihepatitis in liver, a condition not previously reported in the existing literature. Perusal of available literature did not reveal any other published article on pathology of combined aflatoxin and ochratoxin toxicity under natural condition.

In this combined toxicity of aflatoxin and ochratoxins, histological changes mainly observed in liver and kidney. The common findings were congestion of blood vessels and sinusoids, fatty changes. Along with these findings, unique histopathological findings recorded were: 1 case of cirrhotic changes (diagnosed by Masson's trichrome staining), 2 cases of bile canaliculi proliferation (diagnosed by Luna Ishak Staining). The case of liver necrotic area revealed presence of colony of Gram-positive bacteria (modified Brown and Brenn staining). Perusal of available literature did not reveal any such histopathological findings in chicken liver under natural condition of combined toxicity of Aflatoxin and Ochratoxins. Similarly, in kidneys, microthrombosis of blood vessel was recorded in this study. Perusal of available literature did not reveal any other published article on histology of naturally occurring combined aflatoxin and ochratoxin toxicity in poultry.

Fig. 2: Swollen congested tan liver

Fig. 3: Enlarged liver with marked congestion and pale necrotic foci.

Fig. 4: Swollen kidney with prominent lobules

Fig. 5: Congested and enlarged kidney appearing dark coloured.

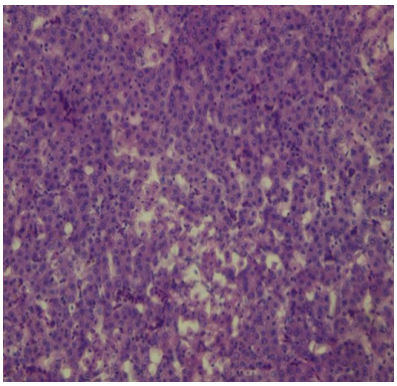

Fig. 6: Liver showing focal area of necrosis (H&E, X 400)

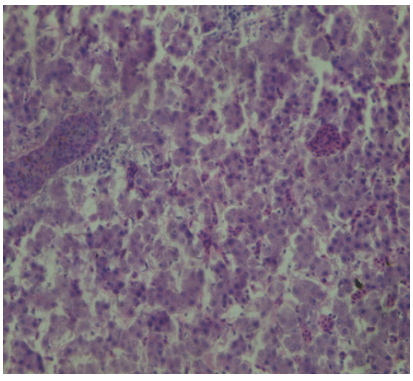

Fig.7: Liver showing congestions, dilated sinusoids and degenerating hepatocytes (H&E, X 100)

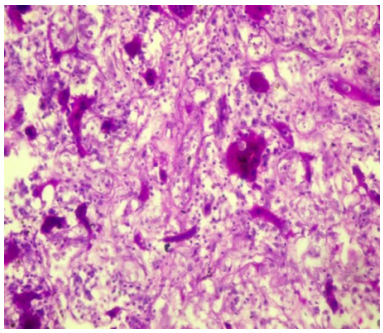

Fig.8: Liver showing proliferation of deep red staining bile canaliculi with fibrous connective tissue (Luna-Ishak. X 400)

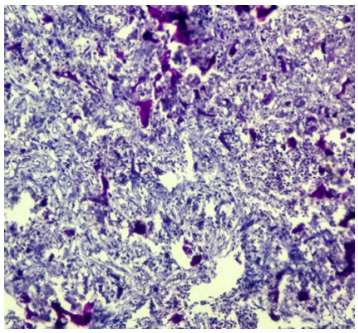

Fig.9: Liver showing extensive proliferation of collagen appearing as blue fibres (Masson's Trichrome, X 400)

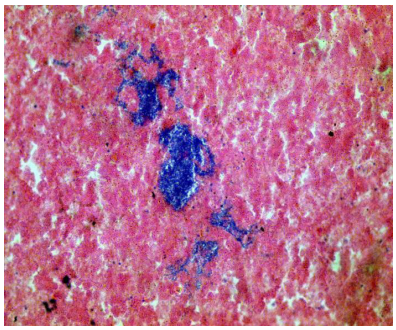

Fig.10: Liver showing an area of coagulative necrosis with presence of bacterial colony (H&E, X 100)

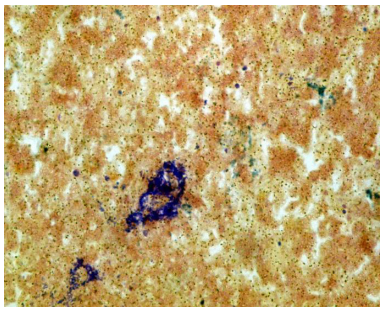

Fig. 11: Liver showing presence of a colony of Gram-positivebacteria (Modified brown and Brenn, X 400)

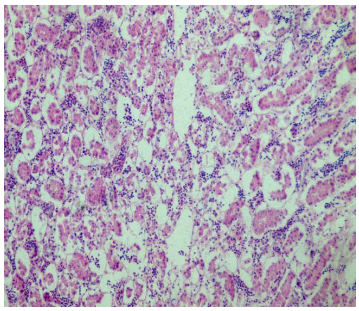

Fig. 12: Kidney showing extensive tubular necrosis and exfoliation (H&E, X100)

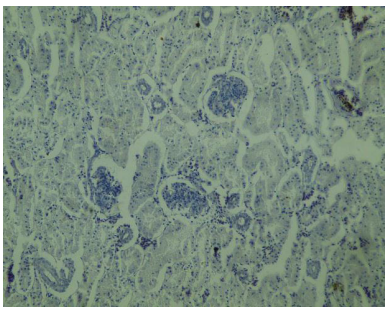

Fig.13: Kidney showing degenerating glomeruli and coagulative necrosis of the cortical tubules (H&E, X 100)

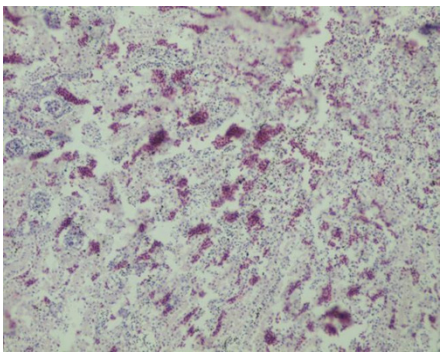

Fig.14: Kidney showing extreme congestion of blood vessel and tubular necrosis (H&E, X100)

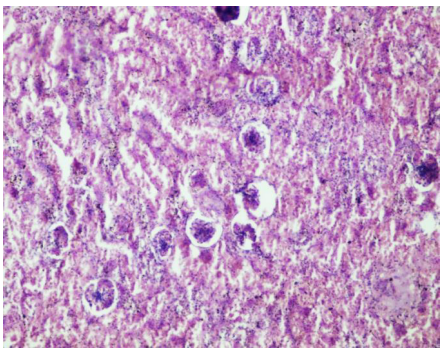

Fig.15: Kidney showing coagulative necrosis in tubules, blood vessel thrombosis, vacuolation in the glomerulus indicating Glomerulopathy (H&E, X100)

Conclusion

The present study confirmed the presence of co-contamination of aflatoxin and ochratoxin in poultry feeds used in the Aizawl, Mizoram region. The affected farms revealed mortality and pathological findings associated with Aflatoxicosis and Ochratoxicosis. 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., & Sardar, R. (2012). Assessment of aflatoxin B1 in commercial poultry feed and feed ingredients. *Journal of Animal and Plant Science,22,* 268-272.

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

Anonymous (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. International agency for research on cancer monoghraph an evaluation of carcinogenic risk to human. Vol-82, pp.33-36, February 12-19, 2002, Lyon, France.

Bancroft, J.D., & 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., & 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), 141-144.

Ceci, E., Bozzo, G., Bonerba, E., Desantis, S., & Tantillo, G. (2008). Ochratoxin A in laying hens: High-performance liquid chromatography detection and cytological and histological analysis of target tissues. *Journal of Applied Poultry Research*, *17,* 151-156.

Hashem, M.A., & Mohamed, M.H. (2009). Haemato - biochemical and pathological studies on aflatoxicosis and treatment in broiler chicks in Egypt. *Veterinaria Italiana 45*(2), 323-337.

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., & 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), 231-241

Khan, S.H., Shamsul, H., Rozina, S., & 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., & Hussain, I. (2010). Pathological effects of aflatoxin and their amelioration by vitamin E in white leghorn layers. *Pakistan Veterinary Journal, 30*(3), 155-162.

Luna, G.L. (1968). Manual of Histologic staining methods. 3rd ed. McGraw-Hill Book Company, New York, USA, pp.93-94.

Parvathi, D., Rajender, R.A., & 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., & Ricelli, A. (2010). Lipoperoxidation affects ochratoxin A biosynthesis in *Aspergillus Ochraceus* and its interaction with wheat seeds. *Applied Microbiology and Biotechnology*, *85,* 1935-194.

Thirumala-Devi, K., Mayo, M.A., Reddy, G., & Reddy, D.V.R. (2002). Occurrence of aflatoxin and ochratoxin A in Indian poultry feeds. *Journal of Food Protection*, *65*(8), 1338-13340.