Research Article

Prevalence and Influences of Mycotoxins on Clinical Aspects, Pathological, and Health Performance of Commercial Layer Flocks in Basrah, South of Iraq

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Abstract | The present study investigated the field incidence of mycotoxin types in hens' feed and their impact on the clinical status, pathological conditions, and health performance of commercial layer flocks in Basrah, south of Iraq. The enzyme linked immunosorbent assay (ELISA) test was used for food samples, clinical signs, gross lesions, and histopathologic examination; all these parameters were used to achieve the aim of the current study. Findings of analysis of feed ingredients (corn, soybean, and mixed feed) after three months of monitoring indicated that there was a lot of contamination, with the majority of the toxins being T-2 toxin, aflatoxins, and fumonisins. Contamination levels showed the highest levels being recorded during the hot month of August. Also, a high degree of variation in ingredient contamination with mycotoxin. Continued exposure to these toxins, even mild levels, resulted in subclinical mycotoxicosis, which caused a reduction in the feed intake, decreased egg production, and cyanosis of the comb and wattles as typical signs. Additionally, diarrhea in some birds indicated a gastrointestinal disorder induced by the local action of mycotoxin. Hepatomegaly, renal discoloration, and proventricular ulcers were found by using gross pathological examinations. The histopathological diagnosis had revealed serious and progressive liver (with hidrotic degeneration, necrosis, and hyperplasia of bile ducts) and kidney (with acute tubular necrosis and interstitial fibrosis) damage. The results highlight that the risk of mycotoxins to the health and productivity of chickens in the area was high and largely undetected. Although mycotoxin binders were used regularly and can cover the clinical symptoms until one or more toxin increased suddenly lead to losses in chickens' health performance. The research concludes that a combined approach of management can be crucial, including the use of strict, continuous food monitoring, better practices of storage, and strategic application of frequent laboratory screening to address this economically devastating problem.

Keywords | Mycotoxin, Flocks, Basrah, Feed, Hens, Pathological

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INTRODUCTION

Mycotoxin is a name first coined in 1960, a combination of two words: Myco, meaning fungus, and toxin, which means poison. Mycotoxins are the poisonous secondary metabolites that are produced by a particular type of fungi that grows on a variety of foodstuffs such as grains, dried fruit, and nuts (Almiman, 2024). Mycotoxins are harmful to animals, such as chickens, which cause a major economic loss in the production of poultry (Alrabadi *et al*., 2018). These toxins are referred to as mycotoxins and are a vast array of toxic secondary metabolites that are mainly produced by the species *Aspergillus, Penicillium*, and *Fusarium*. The aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone are the most significant groups that influence poultry. The groups have varied toxic effects on various organs, which contribute to the hepatic damage, immunosuppression, poor performance, and secondary susceptibility to infections (Filazi *et al*., 2017). T-2 toxin is a very toxic trichothecene mycotoxin, which is produced by *Fusarium* species, usually polluting the cereal grains that are utilized in poultry food (Dalvi, 1986). It acts by its toxicity through inhibition of protein synthesis, resulting in severe impairment of rapidly dividing cells in the gastrointestinal tract and immune system (Sokolović *et al*., 2008). In poultry, exposure to T-2 toxin leads clinically to a decrease in feed consumption and decreased performance. Early detection and close monitoring of the feed quality is important to prevent T-2 toxicosis in poultry farms because it is heat-stable and has persistence in feed (Manafi *et al*, 2012). The most potent aflatoxin of maize and wheat is aflatoxin B1 (AFB1). It is followed by B2, G1, and G2 (Denli *et al*., 2009). Aflatoxins affects the chicken's reducing its growth and feed ratio, producing egg, increase mortality, and condemning carcasses. Aflatoxins cause a variety of metabolic changes, such as hepatic dysfunction, reduced active of digestive enzyme function (Rawal and Kim, 2010). There are many reports of chicken feed contamination with aflatoxins and aflatoxigenic fungi have highlighted and the need to conduct research to identify possibility of preventing aflatoxicosis in the poultry industry (Hussain *et al*., 2008). Clinical signs and pathological lesions, which are related to mycotoxins, are closely connected with the species of poultry, the type of mycotoxin, the amount of mycotoxins ingested, and the time of exposure. Chickens and other animals in field are exposed to a number of mycotoxins and other stressors. Mycotoxicosis signs and lesions may observed in chickens with low concentration of mycotoxins in the diet (Salim *et al*., 2011). Extreme instances of consumption of excess mycotoxins may result in death and a significant reduction in the production of poultry, which is manifested by clinical signs and postmortem changes. Nonetheless, in the majority of instances, mycotoxicosis are chronic conditions induced by the intake of low levels of fungal metabolites, leading to a quantifiable reduction in performance and the manifestation of nonspecific alterations, such as subcutaneous hemorrhage in broilers and immunosuppression (Tritscher *et al*., 2013). In field situations, inferior performance without a clear viral, environmental, managerial factor, or nutritional shortage indicates the potential for mycotoxicosis. In addition to looking at the history, doing clinical and postmortem evaluations of flocks, and looking at tissues under a microscope, it is necessary to test feed for mycotoxins in order to diagnose mycotoxicosis in these long-term instances (Girgis et al., 2010; Bouhet and Oswald, 2005). The poultry industry in Iraq is a big part of the economy and includes many different types of chickens. mycotoxicosis remains a significant issue on chicken farms, particularly in Basrah, and there is a paucity of research on its incidence and the specific toxin involved. The present study aimed to determine the field incidence of different types of mycotoxins in hen feed and to evaluate their impact on the clinical status, pathological lesions, and overall health performance of laying hens in Basrah, southern Iraq.

MATERIALS AND METHODS

Study design and samples collection

The present study was conducted in layer hen poultry houses located in the Al-Zubair district, Basrah province, Iraq. The study duration was 3 months, from June 2023 to September 2023. Poultry farms were monitored for mycotoxin titers and clinical signs of mycotoxicosis, and samples were collected accordingly. Also, the samples included the liver and kidneys were collected from affected chickens in addition, feed samples were collected to monitoring the mycotoxicosis. The samples included corn, soybean and feed mixture. All samples were carefully labeled, and processed immediately for diagnostic evaluation.

Monitoring toxin titer in feed by ELISA

Veratox® for Aflatoxin is an enzyme linked immunosorbent assay kit that uses to accurately and quantitatively measure mycotoxin in a wide range of samples. The test was performed according to the manufacturer's instructions (Veratox®) and also according to the method described in the research (Zheng *et al*., 2005).

Clinical signs

The present study focused on collecting comprehensive data on poultry houses included management, feeding, stocking density, conditions of the storage, flock age, and drug usage, in addition to prior instances of mycotoxin-related concerns.

Gross lesions

Postmortem includes performing examinations of bird specimens that exhibit pronounced clinical signs. The chickens that exhibited clinical symptoms were subsequently euthanized. Visual inspection of all cases was done to identify the presence of gross lesions and viscera change. The gross lesions were prepared as per the procedural steps detailed by Khaleefah and Najem (2021).

Histopathologic examination

Tissue samples from the organs were fixed in 10% neutral buffered formalin and routinely processed. Use appropriate stains, like hematoxylin and eosin, to observe cellular structures and identify histopathological changes (Mohammed *et al*., 2025).

RESULTS

Toxin titers in hens feed

The survival toxicity analysis showed that there was a distinct difference in the amount of toxins in the samples tested. The level of the identified toxin in poultry feed indicated some apparent variation across the studied batches as some samples indicated higher levels of ppb compared to others as show in Figure 1. In addition to this, the toxin levels of soybean samples were variable and the different levels were measured using the ELISA test. Some of the batches of soybean showed high concentrations, which could indicate change in quality of raw materials or in storage as indicated in Figure 2. In the case of corn samples, the toxin levels were also different with some of the levels higher than those of the other feed ingredients. The differences can be seen to be those represented in Figure 3.

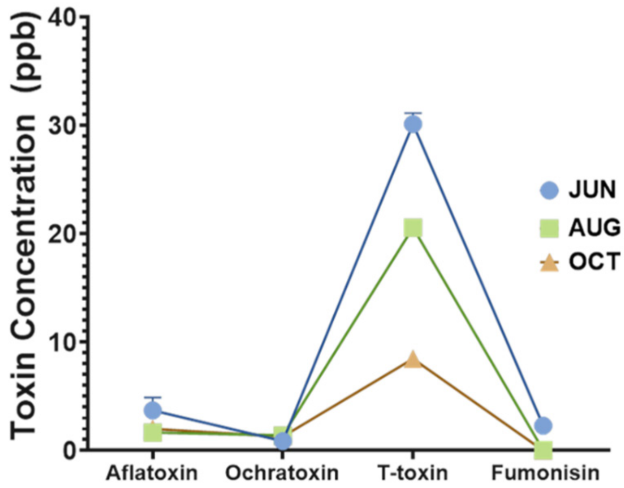

Figure 1: Toxin Concentration in Poultry Feed (ppb): The X&Y and grouped bars diagrams was used to represent the concentration of a toxin contaminant in poultry feed represented as parts per billion (ppb) and measured by using an ELISA test: In that case 1 ppb equals 1 µg of substance per kg of solid (µg/kg) significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test.

Clinical signs

The current study demonstrated that the clinical signs of mycotoxicosis comprised of the decrease in feed consumption, which caused detrimental effect on the health of the birds. In additional to this, decrease appetite can bring other issues in case the situation is not treated. Reduced egg production and eggshell quality are usually the main problems laying hens with mycotoxicosis. Exposure to Mycotoxin, however, has been associated with diarrhea or black feces in some birds (Figure 4A). Also, skin problems in chickens like cyanosis of the comb and wattles (Figure 4B).

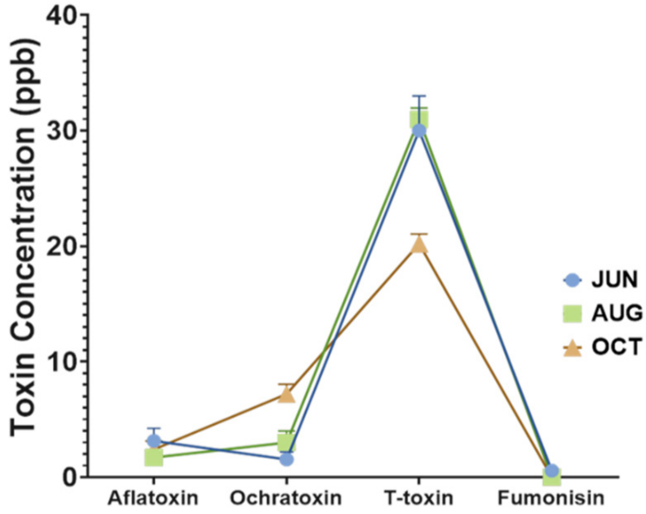

Figure 2: Toxin Concentration in Poultry Soybean (ppb): The X&Y and grouped bar diagrams were used to represent the concentration of a toxin contaminant in soybean represented as parts per billion (ppb) and measured by using an ELISA test: In that case 1 ppb equals 1 µg of substance per kg of solid (µg/kg) significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test.

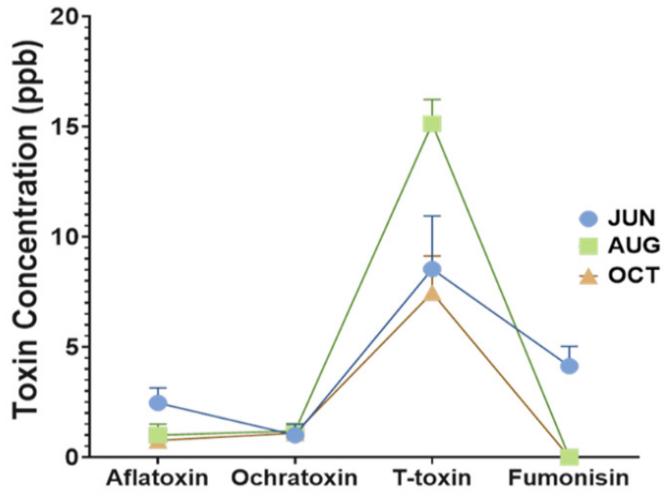

Figure 3: Toxin Concentration in Poultry Corn (ppb): The X&Y and grouped bar diagrams were used to represent the concentration of a toxin contaminant in corn represented as parts per billion (ppb) and measured by using an ELISA test: In that case 1 ppb equals 1 µg of substance per kg of solid (µg/kg) significance (P-value: * < 0.05, ** < 0.01, *** < 0.005, **** < 0.001) was determined by using 1-way ANOVA and post hoc Tukey's test.

Figure 4: Clinical signs associated with mycotoxicosis in chickens. A: Black feces of infected chickens. B: Chicken, shows ruffled feathers, closed eyes, and cyanosis.

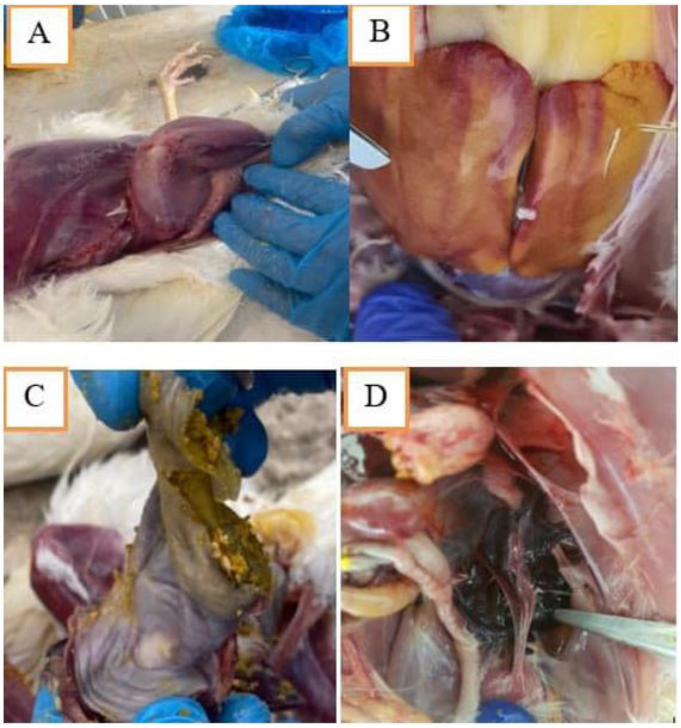

Figure 5: Gross lesions associated with mycotoxicosis in chickens: (A) Enlarged and fatty liver. (B) Muscle abnormalities in chickens: hemorrhagic areas appear as red or brown spots within the muscles. (C) Gizzard with visual abnormalities, such as thickening of the lining, erosions, ulcerations, and changes in color and texture. (D) Enlarged kidneys, with discoloration and hemorrhage.

Gross lesions

The postmortem lesions observed in mycotoxicosis cases can vary depending on the mycotoxin, the affected organ, and the timing and intensity of exposure. The typical pathological abnormalities linked to mycotoxicosis, muscle anomalies in chickens, like Hemorrhagic regions were manifest as either red or brown within the muscles (Figure 5A). Hepatomegaly resulting from hepatic injury and inflammation (Figure 5B). Examination of the gizzard indicated visual abnormalities, such as thickening of the lining, erosions, ulcers, and alterations in color and texture (Figure 5C). Inspected the proventriculus was recorded visible abnormalities, such as changes in color, bleeding, erosion, and ulcers. Kidneys exhibiting a pallid and enlarged appearance. Mycotoxin caused nephrotoxicity, resulting in alterations in both renal dimensions and pigmentation (Figure 5D).

Histopathological changes

Microscopic examination of liver sections observed under the microscope of poultry exposed to mycotoxins showed significant histopathological changes, which indicated that there was severe damage on the liver. The lesions were observed to be hydrostatic degeneration and necrosis of hepatocytes, central veins congestion, and bile ducts hyperplasia, which are the effects of mycotoxins on hepatotoxicity. Figure 6 shows these histopathological changes. Moreover, the histopathological analysis of kidney tissues revealed the evidence of severe renal lesions related to mycotoxicosis. The central changes were, acute tubular degeneration and necrosis, tubular atrophy with casts, interstitial edema and congestion, and thickening of the glomerular basement membranes with interstitial fibrosis. These microscopic observations are shown in Figure 7.

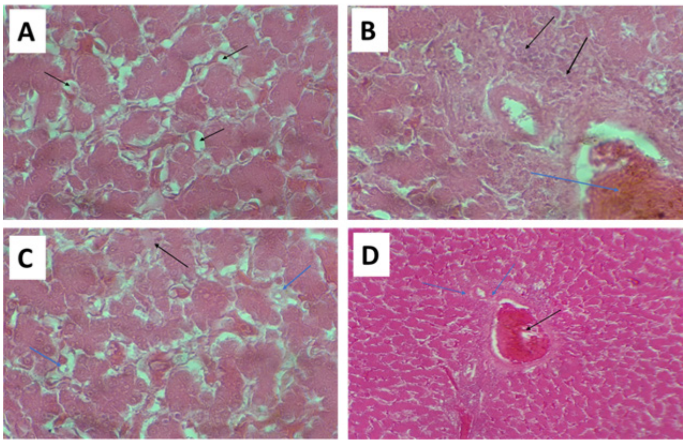

Figure 6: Microscopically, signs associated with mycotoxicosis in poultry: (A) Histopathological section of the liver showing severe hydropic hepatocyte degeneration (black arrows). H&E stain. 400X. (B) Histopathological section of the liver showing hyperplasia in the bile duct (black arrow) and congestion in the central vein (blue arrow). H&E stain. 400X. (C) The histopathological section of the liver shows hydropic degeneration of hepatocytes (blue arrows) and necrosis in the hepatocytes (black arrows). H&E stain. 400X. (D) The liver, which shows congestion in a central vein (black arrow) with hyperplasia in the bile duct (blue arrows). H & Estain. 100X.

DISCUSSION

Contamination of animal feed, processed or compound feed, is common in developing countries. Also, millions of tons of feedstuffs were lost each year due to direct mycotoxin infestation of the world's food grains (Sineque *et al.*, 2017). The present study observed seasonal and ingredient related variations in levels of the major mycotoxins (Aflatoxin, Ochratoxin, T-2 toxin, and Fumonisin) in poultry feed samples (soybean and corn samples) during June, August, and October. August and June are high due to the high temperature to develop toxigenic pathogens, such as Aspergillus, Fusarium, and Penicillium species. Moreover, August is the post-harvest, when grains like corn and wheat are deposited, and this time they are not dried in the most efficient way (Mesterhazy etal., 2022; Esan *et al*., 2024). T-2 toxin was also the highest toxin in all the samples with moderate amounts of Aflatoxin and Fumonisin while the Ochratoxin was lowest concentration whereas, the T-2 toxin has a thermally stable structure that does not break down during feed processing and storage and a high lipophilic structure, which means that it is easily absorbed into the intestinal mucosa and distributed in the body tissues this result agrees with the previous information given (Vörösházi *et al*., 2024; Meneely *et al*., 2023), who reported high prevalence and toxicity levels of trichothecenes in cereal-based diets. In feed samples, the highest concentrations of T-2 toxin and Aflatoxin were observed in corn samples, which indicates that cereal grains are the most favorable environment where fungi reproduce and generate toxins. This result agrees with the research (Álvarez-Días *et al*., 2022), who mentioned that corn and wheat were the most susceptible to aflatoxin contamination compared to oilseeds.

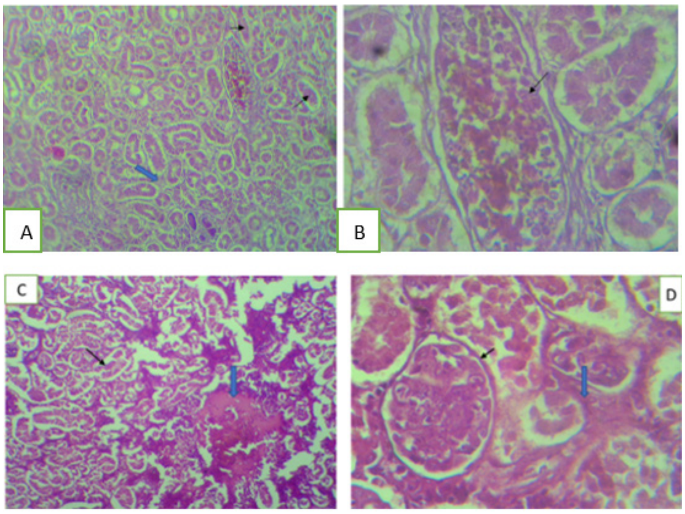

Figure 7: Histopathological section of kidney, A: kidney section showing acute tubular necrosis (black arrows) and interstitial congestion (blue arrow). B: coagulative necrosis of tubular epithelial cells (black arrow) and mild interstitial edema. C: Kidney demonstrating acute tubular degeneration (black arrow), some tubular atrophy formation of tubular casts (blue arrow), and interstitial edema. D: The glomerular basement membranes show signs of thickening (black arrow) and interstitial fibrosis (blue arrow).

The present study showed that the clinical signs of mycotoxicosis included reduced feed intake, which led to harmful consequences on the health and immune state of birds. Moreover, this reduced appetite may lead to additional problems if the condition is left untreated. The present study was similar to that of (Mgbeahuruike *et al*., 2021), which described that mycotoxicosis was associated with feed consumption, and weight gain was low in broiler chickens fed contaminated feed.

Laying hens affected by mycotoxicosis often primarily suffer from reduced egg production and decreased eggshell quality. This result agrees with (Dazuk *et al*., 2020), who reported that mycotoxin consumption impaired the performance and egg quality of hens. However, mycotoxin exposure has been linked to dermatological issues in chickens, such as cyanosis of the comb and wattles (Meneely *et al*., 2023). Also, the present results indicate that macroscopic changes related to mycotoxin consumption induced gastrointestinal disturbances, such as diarrhea or a change in the color of feces. This result agrees with (Li *et al*., 2022), that reported, exposure to the toxin results in adverse pathological consequences in the gastrointestinal tract and in parenchymal tissues such as the liver (the key organ for its metabolism), kidneys, and reproductive organs.

The hepatic and renal foci of the current study indicate the evident pathological course, which can be attributed to the long-term systemic insult of both organs at the same time. The liver sections in the present study presented sinuosity and central vein congestion, hepatocellular vacuolation, hepatocellular necrosis, and hyperplasia of the bile duct, which were indicative of severe hepatotoxicity in the case of mycotoxin poisoning. Such hepatic results are consistent with the past pathology studies, which found periportal necrosis and inflammatory infiltration in the liver of broilers fed on mycotoxin contaminated diets (Almremdhy *et al*., 2024). In addition, the fatty change and degenerative alteration in the current study is in line with previous reports that show that mycotoxins cause disruption of hepatic metabolism and oxidative stress that leads to dose-dependent histopathological lesions of poultry liver tissues (Mehtab *et al*., 2021). The observed coagulative necrosis, hemorrhage, and fibrotic alterations of the studied liver sections also reflect further development of the irreversible hepatic damage. The same mechanisms have been reported in previous reviews, which reported these lesions to be the result of mycotoxin's ability to inhibit protein production, antioxidant defenses, and inflammatory and fibrogenic reactions in the hepatic tissue (Bonerba *et al*., 2024). Similarly, the renal outcome of the current research, such as acute tubular degeneration and necrosis, tubular atrophy with casts, interstitial edema and congestion, and thickening of the glomerular basement membrane with interstitial fibrosis, exhibit severe nephrotoxicity of mycotoxins. These findings are in close accordance with the previous reports that have found tubular and glomerular necrosis, swelling of the kidney, and paleness of kidneys in mycotoxin-exposed broilers (Ayofemi, 2020). Moreover, the persistent renal changes in this report are in line with the experimental and review articles attributing mycotoxin exposure to the gradual degeneration of renal tissue and functional renal deficiency, and this confirms its strong nephrotoxic potential (Okasha *et al*., 2024).

The current study indicates that mycotoxicosis is a prevalent and economically important issue in commercial layer flocks in Basrah, southern Iraq and has a strong connection to the quality of feeds and the composition of raw materials. In laying hens, chronic exposure to low concentrations of mycotoxins causes subclinical mycotoxicosis, which insidiously advances and predisposes the loss of production, immune suppressive effect, and severe pathological changes, especially liver and kidneys. The hepatic and renal lesions that were observed indicate the accumulative and irreversible effect of mycotoxin toxicity even at mild levels. Moreover, adding of mycotoxin binders can delay the diagnosis, and sudden rises in the level of one type of toxin can cause acute significant losses in the hens pereformances.

Based on the findings of present study especially in hot climatic conditions and after the storage of harvests, it is necessary to enhance the practice of storing feeds, regulating moisture and temperature, and rigorously managing the quality control in order to reduce fungal growth and the production of toxins. Furthermore, regular laboratory screening of mycotoxin in feed. The subclinical case of mycotoxicosis needs to be detected early, to limit the pathological damage, avoid production losses, in commercial layers.

CONCLUSION

The study concluded that there was a lot of contamination, with the majority of the toxins being T-2 toxin, aflatoxins, and fumonisins. Contamination levels showed the highest levels being recorded during the hot month of August. Continued exposure to these toxins, even mild levels, resulted in subclinical mycotoxicosis Hepatomegaly, renal discoloration, and proventricular ulcers were found by using gross pathological examinations. The histopathological diagnosis had revealed serious and progressive liver and kidney damage. The results highlight that the risk of mycotoxins to the health and productivity of chickens in the area was high and largely undetected. Although mycotoxin binders were used regularly and can cover the clinical symptoms until one or more toxin increased suddenly lead to losses in chickens' health performance.

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NOVELTY STATEMENT

The novelty of the present study provides that subclinical mycotoxicosis is a prevalent and economically important issue in commercial layer flocks In laying hens, chronic exposure to low concentrations of mycotoxins causes subclinical mycotoxicosis, which insidiously advances and predisposes the loss of production, immune suppressive effect, and severe pathological changes, Results were observed indicate the accumulative and irreversible effect of mycotoxin toxicity even at mild levels. Moreover, adding of mycotoxin binders can delay the diagnosis, and sudden rises in the level of one type of toxin can cause acute significant losses in the hens pereformances.

AUTHOR'S CONTRIBUTION

Sara Salim Mohammad conducted the Immunoassay Investigations work, carried out the laboratory analyses . Isam A. Khaleefah conceptualized , analytical guidance,and prepared the initial manuscript draft. Hussein M. Shadood collected the samples, supervised the generated data, and reviewed the manuscript. Haider Rasheed Alrafas, statistical formatting. Budoor M. Lateif provided guidance and interpretation for the histopatholgical changes and reviewed the manuscript. Harith abdullah najem clinical monitoring and field diagnosis . All authors reviewed and edited the article.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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