MYCOTOXINS IN POULTRY FEED AND FEED INGREDIENTS IN SULAYMANIYAH, KURDISTAN REGION OF IRAQ

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ABSTRACT

Mycotoxins are fungal metabolic byproducts that can contaminate animal feed and human food. To investigate the incidence of mycotoxins in poultry feed in Sulaymaniyah Governorate, Iraq, 173 samples of feed (n =150) and feed ingredients (n = 23) were collected from 49 poultry farms and feed mills. The samples were analyzed to quantitatively determine the existence of five mycotoxins, namely aflatoxin, fumonisin, ochratoxin, T-2, and zearalenone, using specific kits. All the tested samples contained ochratoxin at concentrations ranging between 1.0 and 6.0 µg/kg and averaging 3.4 ± 0.1 µg/kg. About 94.8% of the feed and feed ingredient samples contained a minimum of four mycotoxins in different concentrations. There is a need to understand the cumulative toxic effects better when several mycotoxins occur in animal feed, which may result in issuing new regulations about the maximum allowed concentrations of mycotoxins in feedstuff.

INTRODUCTION

Mycotoxins are secondary metabolites synthesized by specific fungi such as species of Aspergillus, Fusarium, and Penicillium (1). Over 200 species of fungi can produce mycotoxins, and about 300 chemically different fungal metabolites have been identified as mycotoxins. These metabolites can occur in a wide range of raw materials originating from animal and plant sources, which are used in the preparation

of animal feed among the agriculturally-important, mycotoxins, the ochratoxins and aflatoxins are the most catastrophic to the poultry industry (2). Other mycotoxins comprise zearalenone, fumonisins, nivalenol, and T-2, which can negatively impact the health and productivity of poultry farms (3).

Several factors influence the rate of contamination of poultry feed by mycotoxins. These factors include weather conditions, species of fungi contaminating the harvests, method of gathering the crops, seasonal variations, as well as the methods for the storage of provender and ingredients (4). Fungal species may contaminate poultry feed and ingredients, and remain dormant until suitable growth requirements, such as high humidity and aerobic condition, become available. Under desirable conditions, the contaminating fungi start to grow and produce different mycotoxins as secondary byproducts (5).

Mycotoxins may cause detrimental effects on the health and growth performance of poultry, which may involve inhibition of organ functions, immune suppression, reduced egg production, and stunted growth (4, 6). These effects might eventually lead to severe economic losses. Besides, fungal metabolites may also threaten the health of humans that consume contaminated poultry meat. The presence of multiple mycotoxins in human food and livestock provender can exacerbate the toxicity problem through the development of a synergistic effect (7).

Poultry feed is prepared from a variety of ingredients that originate from animals and plants. Cereals are added to poultry feed as a source of energy, while proteins may originate from plant sources, such as soybean and peanut, or animal sources, such as fish and bone meals. These ingredients may be contaminated with mycotoxins such as aflatoxin B1, zearalenone, ochratoxin A, and fumonisins (8). Agencies, for example, the United States Food and Drug Administration and the European Commission, have established regulatory guidelines that determine the maximal tolerated levels of several mycotoxins in food and animal provender. Variations in the limiting concentrations occur according to the type of raw material, species, age of the animal, and intended use (6).

The incidence of mycotoxins in poultry feed has been reported throughout the world (4, 9, 10). The poultry industry in the Governorate of Sulaymaniyah, Kurdistan Region of Iraq, is an essential sector of agriculture that provides food for more than 1.5 million people and supports the region's economy. However, there is little data about the incidence of mycotoxins in animal feed and ingredients. Therefore, this work intended to inquire about several mycotoxins, namely aflatoxin, ochratoxin A, fumonisins, zearalenone, and T-2 in feed and feed ingredients used locally for poultry in Sulaymaniyah. This study provides preliminary data about the magnitude of poultry feed contamination for consideration of other ingredients that are less susceptible to be contaminated by mycotoxins.

MATERIALS AND METHODS

Study area and sampling: Sulaymaniyah is one of 18 governorates of Iraq that is located in the northeast of the country. The governorate consists of 12 districts, namely Chamchamal, Darbandikhan, Dukan, Penjwen, Pishdar, Ranya, Slemani, Mawat, Qaradagh, Said Sadiq, Sharazoor, and Sharbazher (Figure 1). The first seven districts contain 99 poultry farms, which account for the majority of the farms in Sulaymaniyah.

Samples of feed and feed ingredients were collected from several districts in Sulaymaniyah. Farms were visited from January to December 2019 to gather specimens of feedstuff. Some of the poultry farms were visited more than once for sampling. Batches of feed that were recently imported were considered. Larger farms, which had their feed mills in location, were preferably selected, and if several farms were dependent on the same feed mill in a specific locality, samples were collected only from the feed mill to ensure the coverage of a larger area.

Two to four kilograms of feedstuff and ingredients were taken from each farm by smaller portions from several 50-kg capacity bags. Collected specimens from each farm were put in tightly closed plastic bags and stored at -18°C until they were analyzed for mycotoxin contents.

Quantification of mycotoxins in feed and ingredients: Samples of feedstuff and ingredients that were taken from each poultry farm were mixed well, and a

measured amount was ground using a laboratory grinder. Ten grams of the ground specimen was taken and put in a suitable container, and 70% methanol was added at a ratio of 3:1. The mixture was shaken for two minutes and filtered using a Whatmann # 4 filter paper. One milliliter of the filtered solution was put in a 1.5 mL-capacity centrifuge tube, and the tube was centrifuged for one minute. Quantitative determination of each of the five fungal metabolites in the poultry feed was then conducted using different ROSA® WET® Quantitative Test kits from Charm Sciences, Inc. (MA; USA). The names and order codes of the kits used for the quantification of the mycotoxins are shown in Table 1. The instructions of the manufacturer were followed to determine the amount of each mycotoxin. Each test was repeated three times to confirm the consistency of the results.

Analysis of data: The concentrations of the different fungal metabolites in the feed, wheat, and corn samples were compared statistically to determine the most common cause of feedstuff contamination by mycotoxins. The Iraqi climate is characterized by a hot and dry summer and a cold and wet winter. The weather in spring and fall months is fair and characterized by a higher rate of humidity, which favors the fungal growth and development in these seasons. Hence, the concentrations of different mycotoxins were measured and compared according to the season to determine the influence of seasonal changes of weather on the rate of feed contamination by fungal metabolites. Statistical comparison was conducted using one-way analysis of variance (ANOVA), followed by post hoc (Duncan). A probability level lower than 0.05 was considered statistically significant.

Table 1. Names and order codes of the kits used in the quantification of mycotoxins **.**

Mycotoxin	Name of Kit	Order Code
Aflatoxin	FAST5 Aflatoxin Quantitative Test	LF-AFQ-FAST5-100K
Fumonisins	FAST5 Fumonisin Quantitative Test	LF-FUMQ-FAST5-100K
Ochratoxin	Ochratoxin Quantitative Grain Test	LF-OCHRAQ-G-100K
T-2	T-2/HT-2 Quantitative Test	LF-T2-HT2-100K
Zearalenone	ROSA Zearalenone test for Grains and Feeds	LF-ZEARQ-100K

RESULTS

Study area and sampling: Forty-five poultry farms and feed mills from seven districts in Sulaymaniyah, namely Chamchamal, Darbandikhan, Dukan, Penjwen, Pishdar, Ranya, and Slemani, were included in this study (Figure 1). These districts contain the majority of poultry farms and feed mills in the governorate. Initially, it was intended to take a consistent number of samples from each district, but only four samples were taken from the district of Penjwen since the area contains only a few poultry farms. However, the results indicated that the levels of mycotoxins in poultry feed in Penjwen were close to those of other districts (Table 2). The total number of collected samples from January to December 2019 was 173, which included 150 feed, 14 wheat, and nine corn specimens. Of the 173 samples, 26 were gathered in winter, 49 in spring, 77 in summer, and 21 in fall months.

Level of mycotoxins in the collected samples: All the tested specimens contained two mycotoxins at least, and 99 samples comprised all five of the fungal metabolites. Also, 65 samples were positive for 4/5, and 8 of the specimens comprised 3/5 of the mycotoxins. Ochratoxin A was present in all samples at a concentration of 3.4 µg kg-1. Further, T-2 was detected in 170 of the collected specimens at an average concentration of 15.7 µg kg-1. Zearalenone and aflatoxin were detected in 162 and 157 samples, respectively (Table 2). The average concentration of zearalenone in the positive samples was 37.8 µg kg-1, and the concentration of aflatoxin was 4.5 µg kg-

1. Moreover, 119 (68.8%) of the collected specimens contained fumonisins at an average concentration of 1160.0 µg kg-1.Fungal metabolites were detectable in poultry feed in all districts included in our study. The incidences ranged from 58.3% to 100.0% (Table 3). However, the levels of the individual mycotoxins in the poultry farms were not statistically different in the different districts. Further, the concentrations of mycotoxins did not differ statistically ($p > 0.05$) in different seasons (results not shown).

The concentration of mycotoxins in feed, wheat, and corn were also compared statistically, and the results are illustrated in Table 4. Results revealed that the mycotoxin contents of the different specimens did not differ statistically ($p > 0.05$). However, the cumulative concentration of all mycotoxins in feed, corn, and wheat samples averaged at 61.1, 69.2, and 75.4 µg kg-1, respectively. Hence, fungal metabolites were more abundant in wheat than in poultry feed or corn samples tested in our investigation.

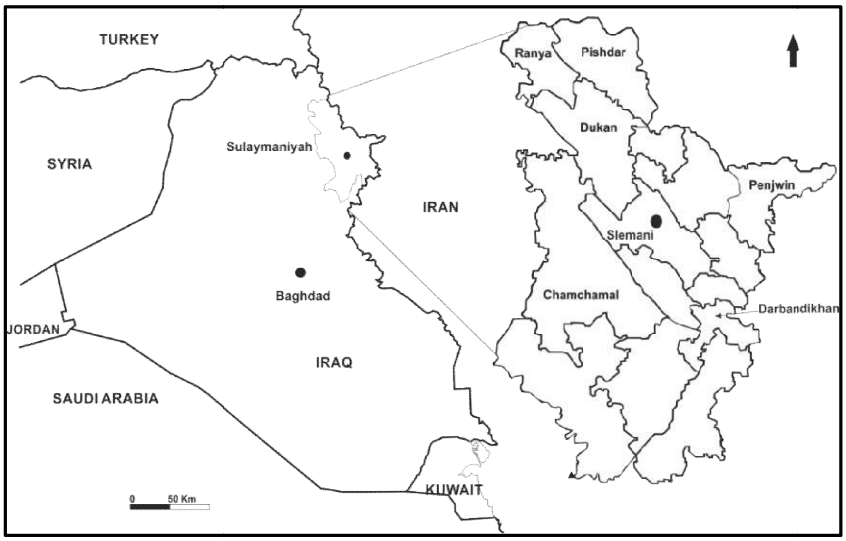

Figure 1. Iraq map, showing the seven districts of Sulaymaniyah, in the northeast of the country, that were included in the study.

Table 2. Mean concentrations of mycotoxins in animal feed in Sulaymaniyah, Iraq

Mycotoxin	Occurrence† (%)	Concentration (µg kg^{-1})	
		Range	Mean
Aflatoxin	90.7	1.0–16.0	4.5 ± 0.3
Fumonisins	68.8	500.0–5000.0	1159.0 ± 84.0
Ochratoxin A	100.0	1.0–6.0	3.4 ± 0.1
T-2	98.3	1.0–80.0	15.7 ± 1.2
Zearalenone	93.6	1.0–180.0	37.8 ± 2.5

Values indicate the percentage of occurrence of the fungal metabolite in 173 samples.

Table 3. Occurrence and concentration of mycotoxins in specimens from seven districts of Sulaymaniyah Governorate.

District	Number of samples	Occurrence (%)					Mean concentration (µg kg^{-1}) ± SE				
		Afl.	Och.	T-2	Fum.	Zea.	Afl.	Och.	T-2	Fum.	Zea.
Chamchamal	40	90.0	100.0	97.5	70.0	100.0	4.6 ± 0.5	3.5 ± 0.1	21.2 ± 3.0	1038.0 ± 165.0	39.0 ± 5.0
Darbandikhan	15	80.0	100.0	100.0	86.7	86.7	5.0 ± 0.9	3.6 ± 0.2	10.4 ± 2.8	1654.0 ± 369.0	52.0 ± 9.9
Dukan	58	93.1	100.0	96.6	58.6	96.6	4.6 ± 0.5	3.2 ± 0.1	17.1 ± 2.1	1187.0 ± 150.0	37.5 ± 4.4
Penjwen	4	75.0	100.0	100.0	75.0	100.0	1.3 ± 0.3	3.5 ± 0.3	9.5 ± 3.7	1083.0 ± 693.0	23.5 ± 9.0
Pishdar	12	100.0	100.0	100.0	58.3	100.0	6.2 ± 1.0	3.5 ± 0.2	12.8 ± 2.4	950.0 ± 152.0	35.0 ± 6.4
Ranya	14	85.7	100.0	100.0	85.7	92.9	3.5 ± 0.6	3.1 ± 0.1	9.7 ± 2.0	1050.0 ± 187.0	37.9 ± 7.7
Slemani	30	93.3	100.0	100.0	73.3	80.0	4.1 ± 0.5	3.7 ± 0.1	13.4 ± 2.9	1116.0 ± 202.0	32.3 ± 6.2

The total number of samples is 173, including 150 feed, 14 wheat, and nine corn samples. Afl. = aflatoxin, Och. =ochratoxin A, Fum. = fumonisins, Zea. =zearalenone

Table 4. Occurrence and concentration of mycotoxins in feed, wheat, and yellow corn in Sulaymaniyah Governorate.

Mycotoxin	Percentage of occurrence*			Mean concentration (µg kg^{-1}) ± SE		
	Feed	Wheat	Corn	Feed	Wheat	Corn
Aflatoxin	90.6	92.9	88.9	4.6 ± 0.3	3.5 ± 0.6	4.8 ± 1.1
Fumonisins	72.0	100.0	33.3	1190.0 ± 90.0	860.0 ± 194.0	850.0 ± 407.0
Ochratoxin A	100.0	100.0	100.0	3.4 ± 0.1	3.4 ± 0.3	3.0 ± 0.3
T-2	99.3	92.9	88.9	15.3 ± 1.3	20.2 ± 4.5	16.4 ± 2.3
Zearalenone	92.7	100.0	100.0	36.6 ± 2.6	41.2 ± 10.6	50.3 ± 10.7

*Percentage of occurrence was calculated based on the total number of each sample type (150 feed, 14 wheat, and nine corn samples). No difference was observed in the concentration of the mycotoxin in the different samples at a probability level of 0.05 (in the same row), using a one-way analysis of variance, followed by Duncan's post hoc.

DISCUSSION

Fungal metabolites are a significant threat to the health and productivity of poultry. Hence, the availability of data about the extent of contamination of poultry feed is a crucial step towards finding ways to reduce the burden of mycotoxins on the health of birds. This study investigated the incidence of mycotoxins in poultry feed in Sulaymaniyah, Iraq, using special kits manufactured by Charm Sciences Inc. These tests are approved by UDSA-FGIS (United Stated Department of Agriculture-Federal Grain Inspection Service) to quantitatively determine the level of each mycotoxin in feed and feed ingredients.

Aflatoxins are metabolic byproducts of Aspergillus flavus and A. parasiticus and are known to be hepatocarcinogenic in humans (11, 12). The risk of contamination of feedstuff by aflatoxins is not only a problem of developing countries. It is estimated that the potential economic losses in the corn industry in the USA due to aflatoxin contamination might reach up to $1.68 billion (13). In Europe, the rate of contamination of maize by aflatoxin has increased in recent years, owing to climate change and global warming (14).

In our study, aflatoxin was found in 90.7% of the samples. The concentration ranged between 1.0 and 16.0 µg kg-1 and averaged 4.5 ± 0.3 µg kg-1. This

concentration is much lower than the recommended maximal allowed levels in feedstuff by the United States' FDA and the EU, which are 300.0 µg kg-1 and 50.0 µg kg-1, respectively (15, 16). However, the probability of feedstuff contamination by aflatoxin is high when conditions are suitable for fungal growth since the vast majority of the samples were positive for this mycotoxin.

Fumonisins are byproducts of Fusarium fungi, including F. nygamai, F. proliferatum, and F. verticillioides. These fungal metabolites cause severe diseases in humans and animals, such as the liver and esophageal cancers (17). The maximum allowed concentration of fumonisin in poultry feed is 20,000.0 µg kg-1, based on the recommendation of the European Union Commission (18). In our study, only one sample of feed contained fumonisins at a rate of 5,000.0 µg kg-1, while the rest of the 119 positive samples contained less than 2,500.0 µg kg-1 of the mycotoxin and averaged 1159.0 ± 84.0 µg kg-1.

Ochratoxin A is a critically harmful mycotoxin produced by A. ochraceus. Research has revealed that ochratoxin is carcinogenic, genotoxic, hepatotoxic, nephrotoxic, neurotoxic, and teratogenic (19). Ochratoxin A is the most harmful of the twenty types of ochratoxins to poultry, causing oxidative stress and many hematological and immunological alterations (2, 20).

The upper limit on this mycotoxin in unprocessed cereals in the EU is 5.0 µg kg-1, under the Codex General Standard for Contaminants and Toxins in Food and Feed (21). Ochratoxin A was detectable in all the assayed specimens in this study, and the concentration ranged between 1.0 and 6.0 µg kg-1 with an average of 3.4 ± 0.1 µg kg-1. Nine of the tested samples (5.2%) contained ochratoxin A at a density of ≥ 5.0 µg kg-1. Comparison between the whole feedstuff, wheat, and corn samples showed no significant differences in ochratoxin A content. In a recent study in the neighboring country of Turkey, from the north of Iraq, ochratoxin A was present in laying hen feed with an average concentration of 27.3 µg kg-1 (22), which means that Fusarium fungi are widespread in the area and might contaminate all cereal products with their metabolic byproducts. Hence, control measures should be taken to reduce the contamination of poultry feed and other cereals by ochratoxin A.

About 98.3% of the samples contained T-2 in different concentrations, between 1.0 and 80.0 µg kg-1. T-2 toxins are a family of more than twenty chemically related compounds produced by several genera of fungi, including Fusarium and Myrothecium. T-2 might cause inappetence, oral and enteral lesions, and impaired immune responses in poultry (23). Destruction of the hematopoietic system, reduced egg production, thinning eggshells, ruffled feathers, and reduced weight gain might also occur as a consequence of T-2 toxicity (24). The overall outcome of T-2 toxicity on several organ systems in poultry is reduced productivity, leading to economic losses in broiler farms. In the European Union, the maximum permitted content of T-2 in feedstuff ranges between 100.0 and 200.0 µg kg-1 (25), while China has restricted the presence of this mycotoxin to 80.0 µg kg-1 (23). None of the samples in the current survey exceeded the maximal allowed T-2 content. However, the fact that 98.3% of the tested specimens were positive for this mycotoxin is an indication that precaution measures are requisite to keep feedstuff free from fungal contamination.

Many species of Fusarium produce zearalenone, such as F. graminearum and F. culmorum. In our survey, zearalenone was detectable in the tested samples at an average concentration of 37.6 ± 2.5 µg kg-1. The European Commission recommends that the maximal limit of zearalenone in cereals and cereal products used as feedstuff is 2,000 µg kg-1 25). The positive samples in this study comprised zearalenone at concentrations ranging from 1.0 to 180.0 µg kg-1, which means that none of the samples contained exceeding concentrations of zearalenone. However, zearalenone was present in 93.8% of the provenders, which implies that contamination of cereals is likely to occur if suitable conditions for fungal growth become available.

The vast majority (94.8%) of feedstuff samples in this study contained at least four of the five tested mycotoxins, including ochratoxin, which is considered one of the most harmful fungal metabolic byproducts. Only 5.2% of the samples contained higher concentrations of ochratoxin than the recommended limits, while none of the other mycotoxins reached the internationally recognized maximal limits in the tested feed samples. However, the cumulative toxic effects of these mycotoxins have not been established yet. Therefore, future research might address studying the synergistic toxic impact of two or more fungal metabolites on the growth and production of

poultry. Understanding the toxic effects of simultaneously administered two or more mycotoxins might result in the emergence of new regulations about the maximal limits of fungal metabolites in livestock feed.

Author contribution: All authors contributed equally in the study design, collection of data, and interpretation of results. HO Dyary prepared the manuscript.

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مسح تقييم السموم الفطرية في علف الدواجن ومكوناتها في السليمانية، إقليم كردستان العراق

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الخلاصة

السموم الفطرية هي نواتج استقلابية فطرية يمكن أن تلوث النظام الغذائي للحيوان والغذاء البشري، وللتحقق من وجود السموم الفطرية في علف الدواجن في محافظة السليمانية، العراق، تم جمع ١٧٣ عينة مقسمة على العلف الغذائي (عدد = ١٥٠) ومكونات العلف (عدد = ٢٣) من ٤٩ مزرعة دواجن ومطاحن علف. تم تحليل العينات لتحديد وجود خمسة أنواع من السموم الفطرية وهي الأفلاتوكسين، الفومونيزين، الاوكراتوكسين، T-2، والزيرالينون، وذلك باستخدام كتات خاصة. كل العينات احتوت على الاوكراتوكسين بتركيز 1.0 الى ٦.٠ ميكروغرام/كغ وبمتوسط ٣.٤ ± ٠.١ ميكروغرام/كغ، احتوت حوالي ٩٤.٨٪ من عينات العلف ومكونات الأعلاف على ما لا يقل عن أربعة سموم فطرية ochratoxin، aflatoxin، T-2، وzearalenone بتركيزات مختلفة وتخلص الدراسة إلى أن توجد السموم الفطرية في علف الحيوانات ومكوناتها في السليمانية، العراق، وهناك حاجة لفهم التأثيرات السمية التراكمية بشكل أفضل عند ظهور العديد من السموم الفطرية في العلف الحيواني، والتي قد تؤدي في النتيجة في إصدار لوائح جديدة حول الحد الأقصى المسموح به من تركيزات السموم الفطرية في الأعلاف.

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