

Analyses of mycotoxins in broiler's local and imported feeds

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Abstract

In the present study 40 samples of broiler feeds that obtained from commercial feed manufacturing company (concerning 21 from Iraqi manufactures and 19 from the Iranian manufactures) were analyzed for the incidence of Aflatoxins, Ochratoxin, T-2, Fumonisin, and Zearalenone mycotoxins. The result has shown that AF was detected in 16 samples 76.19% of the Iraqi manufactures and 16 samples 84.21% from the Iranian manufactures, in concentrations ranging from 1.00-23.00 µg/ kg, and 1.00-2.00 µg/ kg respectively. Prevalence of AF contamination revealed a significant association (P<0.05) between the Iraqi and Iranian manufactures, the highest prevalence was observed in Iranian samples 84.21%, whereas it least in Iraqi samples 76.19%. OCHRA toxin was detected in 21 samples 100% of the Iraqi manufactures and 19 samples 100% from the Iranian manufactures, in concentration ranging from 3.00-4.00 µg/ kg, and (2.00 – 4.00) µg/ kg respectively. prevalence of OCHRA contamination revealed a non-significant (P>0.05) between the Iraqi and Iranian manufactures. T2 toxin was detected in 21 samples (100%) of the Iraqi manufactures and 19 samples (100%) from the Iranian manufactures, in concentration ranging from (2.00 – 16.00) µg/ kg, and (2.00 – 23.00) µg/ kg respectively. Prevalence of T2 contamination revealed a significant association (P<0.05) between the Iraqi and Iranian manufactures FUM3 toxin was detected in 21 samples (100%) of the Iraqi manufactures and 19 samples (100%) from the Iranian manufactures, in concentration ranging from (0.05 – 5.00) µg/ kg, and (0.10 – 2.50) µg/ kg respectively. Prevalence of FUM3 contamination revealed a significant association (P<0.05) between the Iraqi and Iranian manufactures, ZEAR3 toxin was detected in 15 samples (71.42%) of the Iraqi manufactures and 19 samples (100%) from the Iranian manufactures, in concentration ranging from (2.00 – 76.00) µg/ kg, and (1.00 – 41.00) µg/ kg respectively. Prevalence of ZEAR3 contamination revealed a significant association (P<0.05) between the Iraqi and Iranian manufactures, the highest prevalence was observed in Iranian samples (100%), whereas it least in Iraqi samples (71.42%).

Keywords: Mycotoxins, broiler, feed.

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تحليل السموم الفطرية في أعلاف فروج اللحم المحلية والمستوردة

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الخلاصة

أجريت هذه الدراسة لتحليل محتوى السموم الفطرية (Aflatoxins، Ochratoxin، T-2، Fumonisin و Zearalenone في الأعلاف المحلية والمستوردة. أربعون عينة تم تحليلها من أعلاف فروج اللحم التي تم الحصول عليها من ٢١ معمل تصنيعي عراقي و ١٩ معمل تصنيعي إيراني. أظهرت النتائج أن سموم AF قد تم رصدها في ١٦ عينة بنسبة ٧٦,١٩% من المعامل العراقية و ١٦ عينة من معامل الأعلاف الإيراني بنسبة ٨٤,٢١% وبتراكيزات تتراوح بين ١-٢٣ ميكروغرام / كغم و ١-٢ ميكروغرام / كغم على التوالي. كشف انتشار تلوث

AF عن وجود ارتباط معنوي ($P < 0.05$) بين الأعلاف العراقية والإيرانية، لوحظ أعلى معدل انتشار في العينات الإيرانية ٨٤,٢١% بينما أقلها في العينات العراقية ٧٦,١٩%. سموم OCHRA تم الكشف عن وجودها في ٢١ عينة عراقية بنسبة تلوث ١٠٠% وفي ١٩ عينة إيرانية وبنسبة تلوث ١٠٠%. وبتراكيز تراوحت بين ٣-٤ ميكروغرام/كغم و ٢-٤ ميكروغرام/كغم على التوالي. انتشار سموم OCHRA لم يكن معنويًا $P > 0.05$ بين الأعلاف العراقية والإيرانية. أما السموم T-2 فتم الكشف عنها في ٢١ عينة عراقية وبنسبة تلوث ١٠٠% وفي ١٩ عينة إيرانية وبنسبة ١٠٠%، بتراكيز تراوحت بين ٢-١٦ ميكروغرام/كغم و ٢-٢٣ ميكروغرام/كغم على التوالي مع وجود ارتباط معنوي $P < 0.05$. في سموم FUM3 قتم الكشف عن ٢١ عينة علف عراقية بنسبة تلوث ١٠٠% وفي ١٩ عينة علف إيرانية بنسبة تلوث ١٠٠% وبتراكيز تراوحت بين ٠,٥ - ٥ ميكروغرام / كغم و ٠,١ - ٢,٥ ميكروغرام/ كغم على التوالي. مع وجود ارتباط معنوي $P < 0.05$ بين العلف العراقي والإيراني. وفي سموم ZEAR3 تم الكشف عن التلوث في ١٥ عينة علف عراقية بنسبة تلوث ٧١,٤٢% وفي ١٩ عينة علف إيرانية بتراكيز تراوحت بين ٢-٧٦ ميكروغرام/كغم و ١-٤١ ميكروغرام/كغم على التوالي. مع وجود ارتباط معنوي بين العلف العراقي والإيراني، ولوحظ أعلى معدل انتشار في العينات الإيرانية بينما كان أقلها في العينات العراقية ٧١,٤٢%.

Introduction

Mycotoxins are highly poisonous secondary metabolites produced by wide range of fungi principally molds. It's presence in poultry feeds result from the raw material used in their production (1-3). In general, mycotoxins can be produced in various food crops in the field, during processing, transportation and storage when conditions such as temperature and relative humidity are favorable (4) specially in tropic and subtropics region, that the environment comfortable for growth of molds and production of toxins. More than 480 compounds are documented as mycotoxins. Aflatoxins, Zearalenone, Ochratoxin, Fumonisin, and T-2 (5) toxin are some of the mycotoxins and each one will be more or less toxic depending on dose and duration of exposure, which represent an excellent substrate growth and reproduction of numerous fungi. Symptoms of mycotoxins toxicity in birds range from death (6) to chronic diseases, reproductive confusion, immune extinction (7,8) and decreased egg production and meat (9). The effect of toxins on the biochemical-hematological (10), immunological (11), and pathological (12) also been well studied.

The objective of the current study is to compare the level of the 5 major mycotoxins in feed samples of broiler from different factories in Kurdistan Governorate region (KGR)-Iraq and Iran.

Materials and methods

Collecting samples

The study was conducted during (2013 and 2014) in and around Sulaimani province in KGR-Iraq to determine the AF, OCHRA, T2, FUM3, and ZEAR3 in broiler feed. A total of 40 samples of poultry feed were collected from local poultry farms. The samples were homogenized and quartered to obtain a 1 kg of laboratory sample. All collected samples were stored at -20C prior to analysis and thoroughly ground for analysis.

Analysis procedure

The concentration of total Aflatoxins, Zearalenone, Ochratoxin, Fumonisin, and T-2 in the feed samples was determined by using the ROSA system (Charm Biosciences Ins., Lawrence, MA, USA). Fifty gram of grinded feed sample was added to 100 ml of 70% methanol. Then 100 μ l of extract was added to 1 mL dilution buffer, then 300 μ l of the was diluted extract is added to a lateral flow test strip. The test strip was incubated for 10 minutes at 45°C. The test strip was removed and read in the ROSA-M reader.

Data Analysis

Data were analysis using T-test procedures, by SPSS v.18 program to assess the differences between the Iraqi factories and the Iranian Factories.

Results and discussion

In the present study 40 samples of broiler feeds that obtained from commercial feed manufacturing company (concerning 21 from Iraqi manufacture and 19 from the Iranian manufactures) were analyzed for the incidence of AF, OCHRA, T-2, FUM3, and ZEAR3. The total samples number, positive samples, negative samples, and the percentage of the positive samples were presented in the table 1. The Mean \pm S.E., Max, Min, and P value concentration of the contaminated samples by AF, OCHRA, T2, FUM3, and ZEAR3, were represented in table 2.

The result has shown that AF was detected in 16 samples (76.19%) of the Iraqi manufactures and 16 samples (84.21%) from the Iranian manufactures (Table 1), in concentrations ranging from 1.00 - 23.00 μ g/ kg, and 1.00 – 2.00 μ g/ kg respectively. Prevalence of AF contamination revealed a significant association ($P < 0.05$) between the Iraqi and Iranian manufacture, the highest prevalence was observed in Iranian samples 84.21%, whereas it least in Iraqi samples 76.19%. The presence of Aflatoxins in animal feeds causes delayed growth of farm animals and, accordingly, a huge economic loss (13). Some of the

samples exceed the acceptable level according to (14) which mentioned that the accepted level of 20 ppb aflatoxins in poultry feed. It has been well documented that aflatoxin is the most commonly known mycotoxin in poultry feed associated with storage development, but a number of other mycotoxins can also result in adverse effects. In Turkey, 71% of the 52-layer feed samples generally contained <5 ppb aflatoxins, and only two samples exceeded 20n ppb tolerance levels (15). Aflatoxin contamination in poultry feed reported from India and Bangladesh was considerably more than found in our study.

The levels in poultry feed in Bangladesh ranged from 7 to 160 ppb, whereas 60% of the samples of mixed feed found in India contained >10 ppb Aflatoxins (16,17). Other study in Kuwait revealed average aflatoxin concentration in maize at 0.27 ppb (range from 0 to 1.69 ppb), soybean meal at 0.20 ppb (range from 0 to 1.27 ppb), wheat bran at 0.15 ppb (range from 0 to 1.07 ppb), prepared poultry feed for broiler starter at 0.48 ppb (range from 0 to 3.26 ppb), broiler finisher at 0.39 ppb (range from 0 to 1.05 ppb), and layer mash at 0.21 ppb (range from 0 to 1.30 ppb) (18).

Table 1: Occurrence of mycotoxins in poultry feed from local and imported

Toxin	Iraq				Iran			
	Number of samples	Positive samples	Negative samples	% of positive samples	Number of samples	Positive samples	Negative samples	% of positive samples
AF	21	16	5	76.19	19	16	3	84.21
OCHRA	21	21	0	100	19	19	0	100
T2	21	21	0	100	19	19	0	100
FUM3	21	21	0	100	19	19	0	100
ZEAR3	21	15	6	71.42	19	19	0	100

Table 2: Descriptive mycotoxin analyses of broiler feed from local and imported

Toxin	Iraq				Iran				P-value
	Number of samples	Mean ± S.E. µg/ kg	Max. µg/ kg	Min. µg/ kg	Number of samples	Mean ± S.E. µg/ kg	Max. µg/ kg	Min. µg/ kg	
AF	21	3.19±1.23	23.00	0.00	19	0.94±0.12	2.00	0.00	0.003
OCHRA	21	3.52±0.11	4.00	3.00	19	3.63±0.13	4.00	2.00	0.936
T2	21	6.00±0.73	16.00	2.00	19	7.52±1.41	23.00	2.00	0.020
FUM3	21	1.40±0.28	5.00	0.05	19	0.94±0.15	2.50	0.10	0.027
ZEAR3	21	11.14±4.40	76.00	0.00	19	16.42±2.52	41.00	1.00	0.485

OCHRA toxin was detected in 21 samples (100%) of the Iraqi manufactures and 19 samples (100%) from the Iranian manufactures (Table 1), in concentration ranging from 3.00 – 4.00 µg/ kg, and 2.00 – 4.00 µg/ kg respectively. Prevalence of OCHRA contamination revealed a non-significant (P>0.05) between the Iraqi and Iranian manufacture. Ochratoxin, are considered to be mycotoxins that develop during storage. The regulatory limits for Ochratoxin have not been ascertained in United States. European Commission (19) suggested a maximum level of 5 ppb for Ochratoxin A in raw wheat, barley, and rye in international trade as food contaminants. No suggestions have been made for acceptable levels in animal or poultry feed. In another study in Argentina, Ochratoxin A was found in 38% of the poultry feed samples tested range from 25 to 30 (20). Beg *et al.* (18) found in their study in Kuwait, that the highest average concentration of Ochratoxin was found in layer mash feed 9.6 ppb and the

lowest in wheat bran 4.6 ppb. However, the maximum value of >40 ppb was detected in a soybean meal sample.

T2 toxin was detected in 21 samples (100%) of the Iraqi manufacture and 19 samples (100%) from the Iranian manufactures (Table 1), in concentration ranging from 2.00 – 16.00 µg/ kg, and 2.00 – 23.00 µg/ kg respectively. Prevalence of T2 contamination revealed a significant association (P<0.05) between the Iraqi and Iranian manufactures. T-2 toxin are some of the mycotoxins that can significantly impact the health and productivity of poultry species (21). In poultry, the T-2 toxin has been the causative agent for mouth and intestinal lesions in addition to the impairment of immune responses, destruction of the hematopoietic system, declining egg production, the thinning of egg shells, refusal of feeding, weight loss and altered feather patterns, abnormal positioning of the wings, hysteroid seizures or an impaired righting reflex (22).

FUM3 toxin was detected in 21 samples (100%) of the Iraqi manufactures and 19 samples (100%) from the Iranian

manufactures (Table 1), in concentration ranging from 0.05 – 5.00 µg/ kg, and 0.10 – 2.50 µg/ kg respectively. Prevalence of FUM3 contamination revealed a significant association ($P<0.05$) between the Iraqi and Iranian manufactures. Fumonisin is a mycotoxin produced by *Fusarium* spp associated with field contamination and development. This organism is considered present in virtually every seed, and sometimes there is a considerable amount of fumonisin present in symptomless kernels (18). In Taiwan, occurrence of fumonisin in imported maize was examined, and the level in only a few samples exceeded the 0.3 ppm value (23). In a study from Iran (24), fumonisin B1 in maize from two areas was present at mean levels of 3.18 ppm (range from 0.68 to 7) and 0.22 ppm (range from 0.01 to 0.88). In the study of Beg *et al.* (18), >86% of samples tested positive for fumonisin. The average concentration was lowest in soybean meal is 1.42 ppm and highest in broiler finisher feed is 3.2 ppm. These values, however, were, lower than the 30 ppm maximum acceptable concentration for breeding poultry and egg-laying hens.

ZEAR3 toxin was detected in 15 samples (71.42%) of the Iraqi manufactures and 19 samples (100%) from the Iranian manufactures (Table 1), in concentration ranging from 2.00 – 76.00 µg/ kg, and 1.00 – 41.00 µg/ kg respectively. Prevalence of ZEAR3 contamination revealed a significant association ($P<0.05$) between the Iraqi and Iranian manufactures, the highest prevalence was observed in Iranian samples is 100%, whereas it least in Iraqi samples is 71.42%. Zearalenone is another important mycotoxin produced by *Fusarium* spp and is detected along with other mycotoxins in a variety of grains, food, and feed material. It exerts estrogenic effects, and swine appeared to be the most sensitive animal. No regulations have been imposed with regard to the occurrence of this toxin. Weak piglet and litter size have been attributed to the levels of 0.5 to 1.0 ppm dietary zearalenone.

Gajecki (25) recommended a tolerable daily intake of 0.2 mg/kg body weights for animals. Regarding poultry, limited information is available on the adverse effects of zearalenone (26). Zearalenone is receiving serious attention (27) for control, but its regulation poses complexities and needs further insight (28).

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