

Mycotoxins Hazard Assessment of Feedstuffs from Multiple Sources Products Commercially Imported for Iraq

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ABSTRACT

Contamination feedstuffs with fungi or their metabolites toxins poses considerable hazard to public health and animal welfare. The information on mycotoxins identified from a variety of feedstuff in 2021 (soyabean, maize, fish meals, and poultry pellets) was retrieved from archive papers documented in the Centre of Standardization and Quality Control/Department of Agriculture. A total of 1325 samples feedstuff were examined by this Centre using the direct ELISA test to detect aflatoxins, ochratoxin, and trichothecene. The aflatoxin was identified in 67 (5.05%) samples followed by ochratoxin in 52 (3.92%) samples and Trichothecene (T₂) in 50 (3.77) samples. Additionally, the results were showed the co-infection/co-occurrence of mycotoxins from positive samples of soyabean, fish meals, and poultry pellet, although this contamination within acceptable level. Only two samples of poultry feed were determined to have contamination levels above the acceptable limit, as 21ppb with aflatoxins and 19ppb with ochratoxin contamination. The moisture levels were also measured for these commodities by NIR apparatus. From statistics outputs, the squared correlation coefficient (R²) did not share too much variability between moisture and degree of contamination with three mycotoxins identified. The study concluded that mycotoxins remain to be a concern for global health, and their residues in animal feed are important in terms of health safety standards that propose adhering to food chain regulations in commercial markets.

KEYWORDS: Mycotoxins contamination; Risk assessment; animal feedstuffs; Regulations & policy.

الخلاصة

تلوث الأعلاف بالفطريات أو نواتجها السمية يشكل خطراً كبيراً على الصحة العامة ورفاهية الحيوان. تم الحصول على المعلومات الخاصة بالسموم الفطرية والمشخصة في المواد العلفية المستوردة خلال سنة 2021 (فول الصويا، الذرة الصفراء، علف اسماك، علف دواجن) من الارشيف الموثق في مركز التقييس والسيطرة النوعية / وزارة الزراعة في محافظة البصرة. مجموع العينات المفحوصة كانت 1325 عينة من تلك الأعلاف بواسطة هذا المركز باستخدام اختبار ELISA المباشر للكشف عن السموم الفطرية الأوكرااتوكسين والتريكوثيسين. تم تشخيص الأفلاتوكسين في 67 عينة (5.05%) بليها Ochratoxin في 52 عينة (3.92%) و Trichothecene (T₂) في 50 عينة (3.77%)، لكن هذا التلوث كان ضمن المستوى المقبول. أوضحت النتائج ان هناك اصابة مشتركة بسموم تلك الفطريات في فول الصويا والاسماك و علف الدواجن. تم تحديد عينتين فقط من علف الدواجن كانتا بمستوى تلوث أعلى من الحد المقبول، 21ppb في Aflatoxins و 19ppb في Ochratoxin. تم قياس نسبة الرطوبة في تلك الأعلاف بواسطة NIR. اوضحت النتائج الإحصائية ايضاً، ان معامل الارتباط التربيعي (R²) لم يشترك في الكثير من التباين بين قياسات الرطوبة والسموم الفطرية. الدراسة استنتجت ان السموم الفطرية مصدر قلق صحي عالمي، وتعد بقاياها في الأعلاف الحيوانية أمراً بالغ الأهمية ضمن معايير السلامة الصحية التي توصي بالالتزام بالقواعد التنظيمية المتعلقة بالسلسلة الغذائية في الأسواق التجارية.

INTRODUCTION

Agriculture and livestock industry inspection has prioritized animal feed safety in order to protect animal welfare from potential hazards. Mycotoxin is a toxic compound primarily formed by filamentous fungus that grow on food or by products [1]. More than 300 lethal fungal toxins

were identified, posing a risk to animals as well as human health [2]. Fungi belonging to the genera *Aspergillus*, *Penicillium*, and *Fusarium* can produce the most hazardous mycotoxins. Fungi typically infect animal feed cereals such as maize, wheat, barley, and soybean during the preharvest and postharvest periods, when temperature and

humidity are optimal for their growth [3]. Storage conditions, micronutrients, harvest season, and geographical areas are all components that can influence mycotoxins thriving and outspreading in food. Associated with exposure to fungal toxins occurs either directly or indirectly through the consumption of contaminated food commodities containing mycotoxins, such as milk and meat products [4]. Mould mycotoxins can remain stable through food chain process or even in various heat temperatures [5,6].

Contamination of animal feed or food commodities with mycotoxins cause a variety of health problems, depending on amounts of mycotoxins and type of toxins released [7]. Aflatoxins (AF) is the most common toxin produced by the genus *Aspergillus*, has carcinogenic effects in liver after long-term exposure [8,9], and may be cause death from outbreak poisoning [10]. *Fusarium* spp. also produce zearalenone, a mould toxin that can bind to estrogen and reverse its effect on uterus, resulting sterility in animals [11-13]. Moreover, Ochratoxins produced by *Penicillium* and *Aspergillus* cause nephritis and immunosuppression in humans, as well as adverse effects on animal growth or their productions [14,15]. Mycotoxin poisoning in humans cause diarrhea, liver and kidney damage, pulmonary oedema, vomiting, haemorrhagic, and as a chronic form, tumor development probably emerged [16]. Mycotoxins have a range of detrimental effects on animal productivity, leading to weight loss, reduced fertility, impaired immunity, and increased susceptibility to disease infection [17]. Fungi toxins have also resulted in the loss of livestock industry and constrain global trade [18,19]. A widespread of contaminated grains with mycotoxicosis leads to abandonment and damaging harvested crops, which has a substantial economic impact on agriculture entrepreneur. For example, the mean economic annual costs of crop losses were estimated to be USD 932 million due to aflatoxins, fumonisins (FB), and deoxynivalenol [20].

Mycotoxin is a detectable substance, and the Enzyme Linked Immunosorbent Assay, (ELISA) test and High-Performance Liquid Chromatograph (HPLC) are used to evaluate or identify mycotoxins contaminates present in animal feed and commodities [21,22]. The approaches mentioned above are also used in monitoring and

mitigation strategies to determine a degree or tolerant level of contamination of mycotoxins in food, therefore maintaining food safety is importantly necessarily for shielding health [23,24]. The objective of this study is to use documented data of mycotoxins identified in animal feedstuffs for assessment the risk of contamination.

MATERIALS AND METHODS

Collection data

The elicited information on mycotoxins identification from a variety of grains and feedstuffs (Soyabean, Fish-meal, Maize, and Poultry Pellets) was retrieved from archive papers after officially requested from the Centre of Standardization and Quality Control/Department of Agriculture in Basrah, involved the year of 2021. The total number of samples examined by their lab were 1325 samples feedstuff (soyabean, 684; maize, 531; fish meals, 93; poultry pellets, 17). These feedstuffs regularly were exported to Iraq and originated from Argentine, Emirates, Iran, Iraq, Saudi Arabia, and Ukraine. Three types of mycotoxins including Aflatoxins, ochratoxin, and trichothecene were detected by this Centre and reported to entrepreneur/traders, while a copy of these reports stored at the Centre. The direct ELISA test (Enzyme-Linked Immunosorbent Assay) is utilized by their lab and also a moisture for each sample collected was assessed. To access to the stored data, a permission was granted by the office of this Centre. All of the data was entered into an excel spreadsheet and managed for statistical analyses.

Protocol test grain samples

The food samples selected were tested using a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) (Veratox-Neogen product) for the quantitative analyses of three types of mycotoxins included Aflatoxin, Ochratoxins, and Trichothecene. The test aimed to allow the user/traders to obtain the exact safe mycotoxins concentrations in parts per billion (ppb) for all inclusion samples. Briefly, free mycotoxins in the samples and control are permitted to compete with enzymes-labelled fungi toxins (conjugate) for antibody binding site. A microwave reader read the test and calculate concentration of the fungi toxins through the NIR

spectroscopy (Near Infrared) at the wave length 470nm. The moisture of the samples was examined by the NIR application. Based on standardization grain safety and regulation regime, the maximum safely allowed level of concentrations of Aflatoxins should be <20 ppb while Ochratoxins <15 ppb and Trichothecene (T₂) <150 ppb.

Statistical analysis

The statistical package STATA version 14.2 was used to analysis the data. Mean (M), standard deviation (SD), a square correlation coefficient (R²), t-distribution with 95% Confidence Interval (CI), and their p. value were calculated, especially to find out the linear relationship between humidity (as cofactors) with mycotoxins levels.

RESULTS AND DISCUSSION

The number of samples positive to mycotoxins contamination were 74 samples from overall 1325 samples tested during 2021 [5.58% (95CI%:4.4-7.0)]. Figure 1 illustrates the number of samples and types of commodities imported for Iraq, and the countries from where the feedstuffs were sourced. Additionally, compared to maize and poultry pellets, soyabean samples are less contaminated with mycotoxins, even though fish meals seem to be more contaminated. The aflatoxin was identified in 67 (5.05%) of the

samples followed by ochratoxin were found in 52 (3.92%) samples and Trichothecene (T₂) in 50 (3.77) samples (table 1). Soybean, fish meals, and poultry pellets were identified as being positive for co-infection/co-occurrence of three mycotoxins. Only two of the 17 poultry feed samples were tested positive for mycotoxins (21ppb for aflatoxin and 19ppb for ochratoxin), and they were above the acceptable level (table 2). The relationship between moisture and the three identified mycotoxin is shown in Table 3. For all samples positive with aflatoxins, Ochratoxins, and Trichothecene, the R-squared did not share too much variability (0.25, 0.05, and 0.08). The average moisture was calculated as 7.41% with a standard deviation of 1.09.

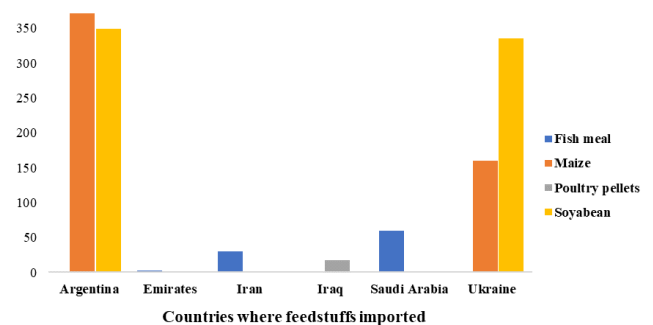


Figure 1. Number of samples from four types of feedstuffs sourced from different countries.

Table 1. Number and proportion of positive animal feedstuffs with three types of mycotoxins.

Type of mycotoxins	Soyabean N (%)	Maize N (%)	Fish meal N (%)	Poultry pellets N (%)	Total N (%)
Aflatoxins	3 (0.43)	11 (2.07)	45 (48.38)	7(41.17)	67 (5.05)
Ochratoxins	3 (0.43)	0 (0)	48 (51.61)	1 (5.88)	52 (3.92)
Trichothecene (T ₂)	3 (0.44)	0 (0)	44 (47.31)	3 (17.64)	50 (3.77)

N=number, %= percentage

Table 2. Types of mycotoxins identified in animal food samples.

Type of food	Aflatoxins		Ochratoxins		Trichothecene (T ₂)	
	Mean ± SD	Maximum	Mean ± SD	Maximum	Mean ± SD	Maximum
Maize	7.06±0.83	8.5	-	-	-	-
Soyabean	6.36±0.15	6.5	0.26±0.30	2.5	3.50±0.60	3.9
Fish meal	5.92±2.62	12.4	1.85±1.16	5.9	6.12±7.69	31.0
Poultry pellets	10.71±8.69	21.10*	1.76±5.84	19.4*	10.8±22.20	71.6

* Maximum permissible level of Aflatoxins <20 ppb; Ochratoxins <15 ppb; Trichothecene (T₂) <150 ppb

Table 3. measures correlation of three mycotoxins with the moisture factor.

Mycotoxins	Pearson correlation	R-squared	p. value	t-distribution (95% CI)
Aflatoxins	0.50	0.25	<0.001*	4.98 (0.7- 0.18)
Ochratoxins	0.22	0.05	0.05*	1.94 (0.00- 0.20)
Trichothecene (T ₂)	0.29	0.08	0.01*	2.59 (0.00-0.05)

*Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The purpose of this study was to determine the types of mycotoxins found in animal feedstuffs freighted to Iraq throughout period of 2021. Mycotoxins are active chemical substances produced by a wide range of fungi, and they inflict the most financial losses in livestock and crop industries. Growth of mould on cereals and grains appears to be influenced by climate and storage conditions, as well as the geographic region where harvested products are considered economically valuable for farmers/entrepreneurs/stockholders [25]. Fungi growth is also influenced significantly by water activity and high temperature. Multiple species of mould can infect grains or cereals, causing production quality to deteriorate [26], especially in tropical and subtropical hotter regions [27]. Because mycotoxins spread through the food chain and concentrate in animal tissues, it is possible to prevent animals from becoming infected by initial detection their food prior to be fed. Over the past years, because the impacts of climate changes globally, and Iraq has impacted remarkably by heat waves and low precipitation, the country experiencing drought which leads to lower agriculture activity and reduction in animal populations [28]. Hence, the country relies massively on international trades to provide consumers and farmers with essential ingredients feed.

The outcomes from the present study indicated the crucial role of the agriculture and livestock department in making decision about accept or reject contaminated feedstuffs with mycotoxins. The laboratory of the department, however, mainly focuses on detection specifically three types of mycotoxins include aflatoxins, ochratoxins, and trichothecene. Findings from the feed samples, 5.58 (74/1,325) were contaminated with mycotoxins, albeit the degree of contaminations mostly were acceptable under regulation policy [29,30]. The limits and regulation of mycotoxins established by the FAO in agreement with 100 countries, with a decision made by exclusion the higher tolerance levels of fungi toxins, especially regulatory limits concerning aflatoxin contamination [31].

Feedstuff samples were tested using the direct ELISA test. The ELISA is trustworthy test and time-consuming test, that enable to emerge good validated results [32]. The principle of the test is based on binding antibody-antigen with the

sensitivity and specificity is above 90%. The test could produce false positive because an affiliation antibody with unspecific antigens in the targeted samples is likely to occur. In the north-western state of Nayarit (Mexico), the research by [33] carried out an ELISA survey to evaluate aflatoxins in 30 raw food selected samples from 10 farms; with the results, 14 of the samples were tested positive for aflatoxins and a high percentage of moisture (0.34% to 0.65%) were attributed to contamination. The detection of mycotoxins can also be performed through using other methodological tools include thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC), although require high purified samples [34,35].

The data obtained show that soyabean has minimum contamination with all three types of mycotoxins 3 (0.43%), followed by maize and poultry pellets. A screening survey for mycotoxins carried out in Rwanda that soybeans harvested crops were shown a low contamination degree [36]. In contrast, substantial levels of contamination with twenty fungal metabolites toxins mostly belong to Fumonisin were identified in Brazilian maize and poultry feed, which proposed their contribution to exacerbate the risk of mycotoxin toxics exposure in animals and humans [37].

Although around 60% of poultry pellets were examined to contaminate with mycotoxins, only two samples were having higher than normal acceptable standard level. A study from the south Africa region indicated the Fumonisin and zearalenone toxins were highly mycotoxins prevalent in 105 poultry feed using HPLC, with significant intolerance contamination degrees observed [38]. In Croatia, the main reason of increase mortality poisoning in poultry flocks were due to exposure to trichothecene in their feedstuffs [39]. Our hypothesis is that the poultry feed may contain high levels of proteins, vitamins, and minerals that are conducive factor to growth mould. On other hands, the moisture for all feedstuffs was measured at average 7.41 and the correlation coefficient (R^2) was very small and did not show high variability between factor moisture and agents. The low moisture in the feedstuffs may be ascertain an excellence storage condition after harvest, where grains or other fresh commodities preserve in polyethylene sacks which can prevent from intensive contamination with mould [40,41].

In the present study, the co-infection/co-occurrence to more than one mycotoxin was found predominantly in soyabean, fish meals, and poultry pellets. [42] conducted a survey on 330 samples of feed ingredients and found that 60% of the maize samples tested positive for more than one mycotoxin, where AF and FB were the most dominated fungi-toxic co-occurrence. An investigation survey was conducted between 2006 and 2007 in maize fields from Germany, identified around 14 *Fusarium*-related mycotoxins, and deoxynivalenol and zearalenone were detected up to percentage of 90% in maize harvest crops during wet season [43]. However, strategies for control mycotoxins or reduce detoxification in feed includes sorting and separation, washing, solvent extraction, heating, irradiation, and adsorption [44-46]. Thermal treatment can reduce the moisture in the feedstuff while treatment with adsorptive compounds can rapidly detoxify mycotoxins in feed, such as aluminum silicates and Cholestyramine [47,48].

CONCLUSIONS

Mycotoxins have negative effects on human health and substantially can decrease animal productivity. Also, fungus growth on crops causes significant economic losses concerning agriculture as well as animal production. The growth of the fungus is influenced by high temperature and relative humidity conditions. The current study provided insightful details on the function of the agricultural and livestock departments in identifying mycotoxins before making them accessible to feed animals. The evidence from the documents indicated low contamination level of mycotoxins in feedstuff sampled, and this could be attributed to preventative measures adopted for preservation feedstuff after harvest and during transportation. The feedstuffs in the silos in Basrah and other governorates need be investigated for presence mycotoxins, as well as derived animal products in wet markets also need to be monitored to ensure food safety.

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