

# Safety and wholesomeness of ready-made feed used in some broiler farms in Tartous Governorate, Syria



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## ABSTRACT

This study aimed to evaluate the Safety and wholesomeness of broiler feeds by identifying *Aspergillus species*, and detecting contamination with total aflatoxins in both starter (mashed) and finisher feed used to feed poultry (broilers). Samples were collected from six different farms in the Tartous Governorate - Syria, during the period from September 2023 to January 2024. The samples were grown in the laboratory on Potato Dextrose Agar (PDA) culture. Feed samples were analyzed for total aflatoxin content using a high-performance liquid chromatography (HPLC) device. Four species of *Aspergillus* fungi were identified in the studied feed samples: *Aspergillus terreus* (60%), followed by *A. niger* (37.1%), *A. flavus* (1.9%), and *A. nidulans* (1%), as it was found that *A. terreus* was the most prominent species isolated from starter (mashed) feed with a frequency of (96.6%), while *A. niger* was the most prominent species isolated from finished feed with a frequency of (78.7%). The results of determining total aflatoxins from the studied samples showed that three samples of starter (mashed) feed (50%) and four samples of finished feed (66.7%) contained aflatoxins at an average of 0.7 µg/kg and 1.2 µg/kg in the starter (mashed) and finisher feed, respectively. This study concludes that the ready-made broiler feed used in the studied farms was contaminated with different types of *Aspergillus spp.* fungi, and with low levels of total aflatoxins, which poses a threat to the poultry industry due to toxins produced by some fungal species, due to their cumulative effect.

**Keywords:** Broiler, Total Aflatoxins, Starter (Mashed) Feed, *Aspergillus*, Finished Feed

## INTRODUCTION

Securing feed is the most expensive aspect of animal production projects, as it ranges from 60-70% of the total cost. Securing good feed requires protecting it from contamination and spoilage in order to maintain its specifications and quality, by taking appropriate measures in the various stages of feed manufacturing, starting from Field source until the time it is introduced to birds. The environmental conditions for storing grains and feed ingredients are among the most important factors affecting the level of fungal contamination, especially since the feed contains various nutritional substances and added enhancers such as minerals and vitamins encourage the growth of most living organisms (Al-Mihi, 2014), therefore poultry nutrition depends on feeds that constitute a good, suitable environment very important for the growth and reproduction of fungi (Abo-Shama, 2015). Fungal species belonging to the genus *Aspergillus spp* are widespread; Its growth requires a relative humidity of 12% in the grain, and the ambient temperature exceeds 7°C, in addition to the availability of oxygen and energy. *Aspergillus* fungi are among the most common types of fungi that It causes feed spoilage, reduces food quality, and affects animal health (Abdalabbas *et al.*, 2011). The genus *Aspergillus spp.* among the most concerning fungal genera in the world, some fungal species belonging to the genus *Aspergillus spp.* can infect animal tissues (Richard, 2007). Many cases of aspergillosis infection in poultry have been recorded, including infections in broiler chicks in the central, northern and southern regions of Syria, where some species of the *Aspergillus* fungus were isolated from the lungs and air sacs of the studied broiler chicks (AL-Daouad *et al.*, 2011).

The process of converting raw ingredients into compound poultry feed is considered more dangerous, as contamination of poultry feed with mycotoxins (Aflatoxins) is common and widespread. Numerous studies have shown the ability of some species of the *Aspergillus* fungus to produce mycotoxins (Aflatoxins) in poultry feed. Aflatoxins are related to economic standards, as the presence of aflatoxin in poultry feed components affects farm production, including bird production indicators, and at the same time risks introducing these toxins into human food (Ráduly *et al.* 2020). Aflatoxin was detected in five samples of poultry feed in Nigeria, and when conducting fungal isolation, the fungus *Aspergillus flavus* was dominant (Kehinde *et al.*, 2014). In

Iran, a study was conducted to evaluate aflatoxin strains in poultry feed. The study showed that among 54 isolates it was identified as *Aspergillus spp. Flavi* section, 20 isolates (37%) were found to produce aflatoxin (Ghaemmaghami *et al.*, 2020). The Food and Agriculture Organization estimated (FAO) that up to 25% of food crops, and a large proportion of animal feed in the world, are contaminated with mycotoxins (Streit *et al.*, 2013). A global mycotoxin survey in 2013 showed that 81% of nearly 3,000 grain and feed samples analyzed contained at least one type of mycotoxin, which is higher than the 10-year average (from 2004 to 2013) of 76% of a total of 25,944 samples (Murugesan *et al.*, 2015). The results of the Global Mycotoxin Survey in 2019 also indicated that about 20% of poultry feeds contain aflatoxin, and about 5% of them exceeded the regulatory limits of the European Union in different geographical regions according to the Global Mycotoxin Survey (Gruber-Dorninger and Schatzmayr, 2019).

Many studies have proven the prevalence of *Aspergillus* species in poultry feed. In Nigeria, one study revealed the presence of a variety of *Aspergillus* fungi: *A. fumigatus*, *A. parasiticus*, *A. flavus*, *A. niger*, *A. terreus* (Habib *et al.*, 2015), and in Iran, one study showed the presence of some species of *Aspergillus* fungi in poultry feed: *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, and *A. ochraceus* (Ghaemmaghami *et al.*, 2016).

Many previous studies indicate the presence of fungal species belonging to the genus *Aspergillus spp* in poultry feed, which may lead to diseases in poultry such as aspergillosis, or potentially be toxic, and therefore these species can be considered a threat to the health of poultry and humans, as there are no Local studies on the diagnosis of *Aspergillus* species in ready-made poultry feed.

Therefore, this study aimed to evaluate the Safety and wholesomeness of broiler feeds through: diagnosing *Aspergillus* species belonging to the genus *Aspergillus spp*, and detecting contamination with total aflatoxins in ready-made broiler feeds from some farms dedicated to the care and production of broiler chickens.

## MATERIAL AND METHODS

The study was conducted based on methods implemented in previous studies in the same field.

### Sample collection:

Twenty-four samples of broiler feed were collected (12 starter mashed feed samples and 12 Finisher feed samples). Among the collected samples, 12 samples (6 starter (mashed), 6 finisher) were allocated for fungal isolation, while another 12 samples (6 starter (mashed), 6 finisher) were allocated for the determination of aflatoxins. This study was conducted during the period from September 2023 to January 2024 where samples collected from six farms designated for raising broilers in Tartous Governorate. Identical quantities were taken randomly from starter (mashed) feed bags stock. After mixing them, one final sample weighing 500 g was taken and kept in a transparent plastic bag attached to the sample card. Another sample was taken in the same way from the finished feed bags used in each of the studied farms, then transferred to the laboratories of the Faculty of Agricultural Engineering at Tishreen University and stored in the refrigerator at 7°C until use.

### Fungal isolation:

The process of fungal isolation was carried out on a solid general nutrient culture medium, which is Potato Dextrose Agar (PDA), where the contents of each sample were remixed individually, in succession, in the isolation room, and only 1 g of it was taken into a 50 ml numbered Erlenmeyer containing 9 ml of sterile distilled water. The resulting suspension mixture was mixed well with a glass rod for five minutes to obtain a concentration of  $10^{-1}$ , then diluted again by taking 1 ml of the solution and adding 9 ml of sterile distilled water to it to obtain a concentration of  $10^{-2}$ , and in the same way to obtain a concentration of  $10^{-3}$ . For each treatment (concentration), three glass petri dishes (replicates) with a diameter of 9 cm were allocated. Each replicate contained the nutrient culture medium with a thickness of 2 mm. 1 ml of each concentration was grown on the surface of the nutrient culture for each replicate. Three petri dishes were allocated for the control treatment in the same manner, where 1 ml of sterile distilled water was grown on the surface of the culture medium in each.

### Incubation and monitoring:

All replicates of the treatments were incubated in the dark at a temperature of  $25 \pm 2^\circ\text{C}$  and monitored for 10 days until the fungal colonies appeared and differentiated. Then, they were examined to identify the species, and their number was calculated using a set of macroscopic and microscopic morphological features (shape, size, texture, color of the colony, crescents, shape of

the sporophores, and the shape and size of the vesicle and the spores), according to standard methods and approved taxonomic keys (Samson *et al.*, 2010; Pitt and Hocking, 2009 and Nyongesa *et al.*, 2015). The experiment was repeated with an interval of one month in between, and the percentage frequency was calculated through the following equation: (Saleemi *et al.*, 2010)

$$\text{Percentage frequency \%} = (\text{Number of Isolates of one Species} / \text{Total Number of Isolates of All Fungi}) \times 100$$

#### Determination of total aflatoxins:

A high-performance liquid chromatography (HPLC) device (SHIMADZU), made in Japan, was used. HPLC-FLD (fluorescence detector) technology was used, and the carrier phase was used, which is a mixture of distilled water: methanol: acetonitrile (60:20:20) ml, at a flow speed (1ml/min), in addition to a C18 chromatographic separation column (MN, MACHEREY- NAGEL, made in Germany; 150 × 4.6 mm I.D., particle size 5 µm), the wavelengths of the FLD fluorescence detector were: excitation wavelength 365 nm, and emission wavelength 435 nm (Raheb *et al.*, 2020).

The feed samples were analyzed and examined for total aflatoxin content using a high-performance liquid chromatography (HPLC) device, where (25 g) ground sample was placed with (5 g) sodium chloride (granules) and 60% methanol in a blender for 5 minutes, then the sample was filtered with glass filter paper. Then a (4 ml) filtrate was taken and (12 ml) buffer solution (8.5 g sodium chloride "NaCl", 1.91 g sodium dihydrogen phosphate " Na<sub>2</sub>H<sub>2</sub>P<sub>2</sub>O<sub>7</sub>", 0.38 g potassium dihydrogen phosphate " kh<sub>2</sub>Po<sub>4</sub>") was added to it, then the (16 ml) solution (4 ml sample and 12 ml buffer solution) was passed over an extraction column. Aflatoxin, then (15 ml) of the buffer solution is passed over the extraction column, then (1 ml) of methanol is passed and kept, and (1 ml) of methanol is added to it, then (2 ml) are taken and passed over a 0.45-micron filter, then we inject a fluorescence detector into the HPLC device (Raheb *et al.*, 2020 and Almeida *et al.*, 2012).

#### Data analysis:

The SPSS v25 program was used to analyze the data. The Independent Samples T-Test was conducted with the aim of verifying the significance of the significant differences between the average results of the studied elements for the raw and manufactured feed samples (George and Mallery, 2018).

## RESULTS

#### Fungal isolation results:

The results of this study showed the fungal species of *Aspergillus spp* isolated from ready-made broiler feed samples taken from farms dedicated to raising broilers. Where 105 isolates of *Aspergillus* fungi were obtained (58 isolates from starter (mashed) feed, 47 isolates from finished feed) belonging to four species of *Aspergillus* fungi. The T-test for independent samples showed that there were significant differences (P < 0.05) between the means of the fungal species isolated from the tested samples of starter (mashed) and finisher broiler feeds.

It was also found that *Aspergillus terreus* was the most prominent species isolated from starter (mashed) feed with a frequency of (96.6%), while *Aspergillus niger* was the most prominent species isolated from finished feed with a frequency of (78.7%).

In general, the types of *Aspergillus* fungi isolated from the studied feed samples were: *Aspergillus. terreus* (60%), followed by *Aspergillus. niger* (37.1%), *Aspergillus. flavus* (1.9%), and *Aspergillus. nidulans* (1%)., (Table 1 and 2), (Figure 1 and 2).

**Table 1.** Statistical descriptions of fungal species belonging to *Aspergillus* isolated from starter (mash) and finishing feed for broilers

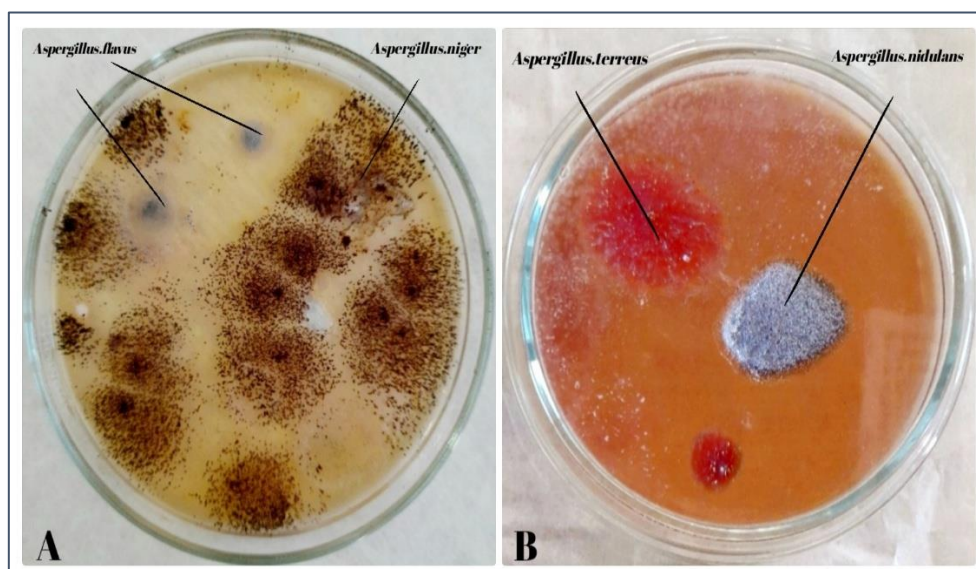
Types of <i>Aspergillus spp</i>	Starter (Mashed) Feed (n = 6)				Finished Feed (n = 6)			
	Isolates			Fr %	Isolates			Fr %
	Range	Mean	Total summation		Range	Mean	Total summation	
<i>Aspergillus .niger</i>	0 - 1	0 <sup>a1</sup>	2	3.4	2 - 13	6 <sup>a2</sup>	37	78.7
<i>Aspergillus. flavus</i>	0	0 <sup>b1</sup>	0	0	0 - 2	0 <sup>b2</sup>	2	4.3
<i>Aspergillus .terreus</i>	0 - 50	9 <sup>c1</sup>	56	96.6	0 - 4	1 <sup>c2</sup>	7	14.9
<i>Aspergillus .nidulans</i>	0	0 <sup>d1</sup>	0	0	0 - 1	0 <sup>d2</sup>	1	2.1
<b>Total</b>			<b>58</b>	<b>100</b>			<b>47</b>	<b>100</b>

<sup>a, b, /1,2</sup>: Levels of significant differences between the means of fungi for the starting (mashed) and finisher feed at the 5% level; n; Number of samples; Fr: Frequency percentage

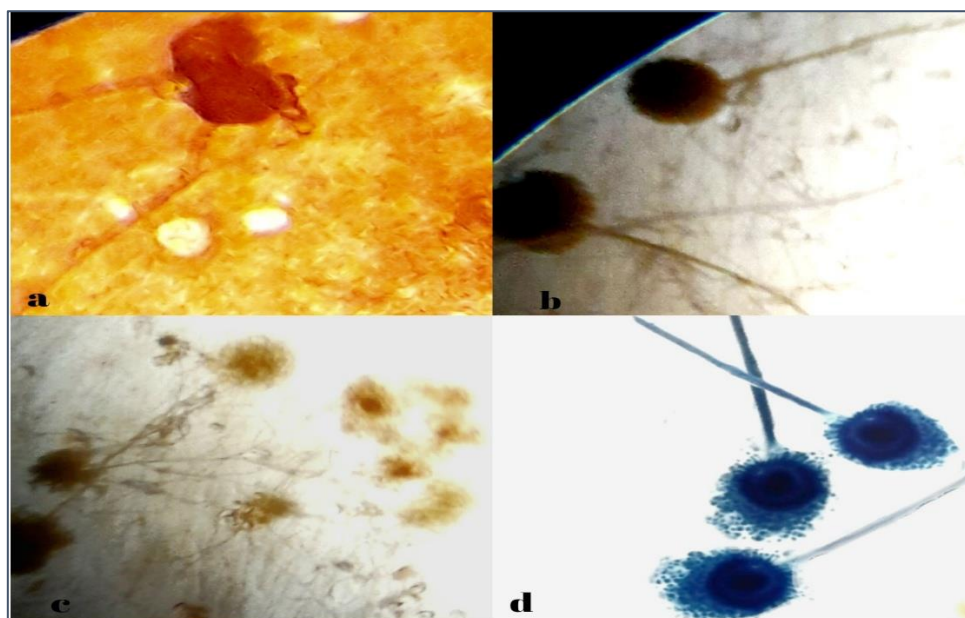
**Table 2.** Total frequency of *Aspergillus* fungi isolated from broiler feed

Types of <i>Aspergillus</i> spp.	n= 12	Total Fr %
	Total number of isolates	
<i>Aspergillus .niger</i>	39	37.1
<i>Aspergillus .flavus</i>	2	1.9
<i>Aspergillus .terreus</i>	63	60
<i>Aspergillus .nidulans</i>	1	1
<b>Total</b>	<b>105</b>	<b>100</b>

n: Total of samples; Fr: Frequency percentage



**Fig. 1.** Fungal colonies of isolated species of the genus *Aspergillus. spp* (in Petri dishes)  
(A): *A. flavus*; *A. niger* (B): *A. terreus*; *A. nidulans*



**Fig. 2.** Sporophores of isolated species of the genus *Aspergillus. spp* (under the microscope)  
(a): *A. terreus* (b): *A. niger* (c): *A. nidulans* (d): *A. flavus*

#### Results of determination of total aflatoxins:

**Table (3)** shows the results of the total aflatoxin concentration in the ready-made broiler feeds, where the total aflatoxin concentration ranged between 0 - 2  $\mu\text{g}/\text{kg}$  and an average of 0.7  $\mu\text{g}/\text{kg}$  in the starter (mashed) feed, while it ranged between 0 - 3  $\mu\text{g}/\text{kg}$  and an average of 1.2  $\mu\text{g}/\text{kg}$  in finished feed; The T-test showed that there

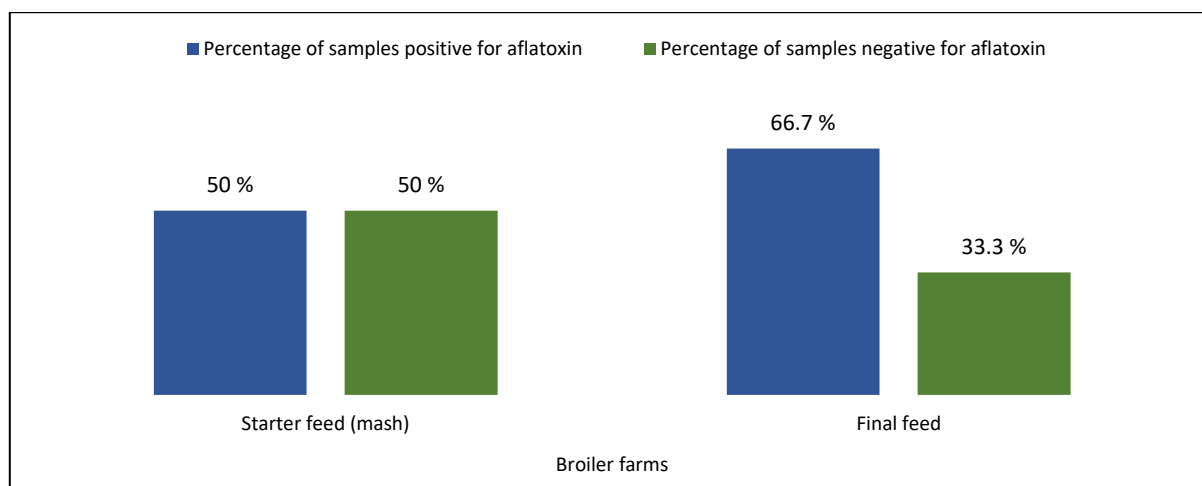
were no significant differences ( $P > 0.05$ ) between the average concentration of aflatoxins in the starting (mashed) and final feed samples.

It was also found that three samples of starter (mashed) feed (50%) and four samples of finished feed (66.7%) contained aflatoxin, i.e. a total percentage (58.3%) of 12 samples taken from broiler farms, while three samples of starter (mashed) feed (50%), and two samples of finished feed (33.3%) were negative for aflatoxin, i.e. a total percentage (41.7%) of 12 samples taken from broiler farms (**Figure 3**).

**Table 3.** Results of the concentration of total aflatoxins in samples of ready-made feed for broilers from the studied farms

Sample source	Sample	Number	Total aflatoxin concentration ( $\mu\text{g}/\text{kg}$ )		
			minimum	Maximum	Average $\pm$ SD
Broiler farms	starter (mashed) feed	6	0	2	0.7 <sup>a</sup> $\pm$ 0.81
	finished feed	6	0	3	1.2 <sup>a</sup> $\pm$ 1.16

<sup>SD</sup>: standard deviation; <sup>a, b</sup>: Levels of significant differences between the means of aflatoxin for the starting (mashed) and finisher feed at the 5% level.



**Fig. 3.** Percentage of total aflatoxins in the studied samples

## DISCUSSION

Two studies in Nigeria showed that the fungus *Aspergillus. Fumigatus* was most frequent in feed used in poultry farms (Habib *et al.* 2015; Aliyu *et al.*, 2016), which is in contrast with the results of this study. While one study noted that *Aspergillus* species were more frequent in starter (mashed) feed and less frequent in finished feed (Ghaemmaghani *et al.*, 2016), and this is consistent with the results of this study, while other studies revealed that fungi belonging to the genus *Aspergillus spp.* they were more common in finished feed compared to starter (mashed) feed (Khosravi *et al.*, 2013; Greco *et al.*, 2014 and Ghaemmaghani *et al.*, 2018).

The reason for contamination of starter (mashed) feed with species belonging to the genus *Aspergillus* may be due to the failure to dry corn well during agricultural and transportation operations, in addition to inappropriate storage, and weather conditions (Ghaemmaghani *et al.*, 2016), while the reason for the frequency of these species in finished feed is due to their ability to survive after heat treatment, moisture, flies, insects, dirty machinery, and air also play major roles by allowing fungal pathogens to reach the feed (Ghaemmaghani *et al.*, 2018).

Three types of poultry feeds (starter, grower, and finisher) from poultry farms in Pakistan were evaluated, and the percentage of positive samples for aflatoxin contamination was 92.5%, and grower feed had the highest frequency of aflatoxin-positive samples (Naveed *et al.*, 2022). In Iraq, twenty samples of starting and finishing feed for broiler chickens were collected to detect aflatoxin residues. The results showed that 3 (15%) of the feed samples were positive, while 17 feed samples (85%) were negative for aflatoxin residues (Khalaf *et al.*, 2015). A global survey of aflatoxin levels showed that the average aflatoxin concentration in the Middle East was 2.4  $\mu\text{g}/\text{kg}$  (Gruber-Dorninger and Schatzmayr, 2019); These data are consistent with the results of this study in terms of low aflatoxin levels in the studied feed samples.

The mean level of aflatoxin in finished feeds has been shown to be higher than starter (mashed) feeds in Iran (Ghaemmaghani *et al.*, 2020); This is in line with the results of this study.

The reason feed samples taken from broiler farms are contaminated with aflatoxins is due to placing feed bags on the ground, which increases the moisture content, while the pressure exerted by the feeds on each other reduces the sizes of feed particles, which increases the risk of contamination with aflatoxins (Munthali *et al.*, 2016).

Contamination of finished feeds with aflatoxins more than starters can be attributed to the fact that heat treatment of finished poultry feeds cannot affect the fungal conidia, the variable resistance of the fungal aflatoxin strain, and thus may lead to the production of aflatoxins if present (Ghaemmaghami *et al.*, 2020).

## CONCLUSION

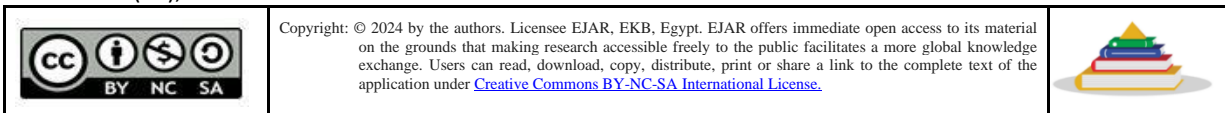
The data and results of this study show that ready-made broiler feed is contaminated with different types of *Aspergillus spp.* fungi, and with low levels of aflatoxins; Also, some species continue to form spores despite being exposed to heat, and this is a matter of concern, as it portends poor health conditions in poultry farms. This not only reduces the nutritional value of feed, but also poses a threat to the poultry industry due to the toxins produced by some fungal species. This study recommends biosecurity measures to improve feed quality and hygiene, use of properly dried grains, and binders that inhibit fungal differentiation and reduce mycotoxins in poultry feed.

**Conflict of Interest:** The authors declare no conflict of interest.

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## سلامة وصحة الأعلاف الجاهزة المستخدمة في بعض مزارع الفروج في محافظة طرطوس، سورية

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هدفت هذه الدراسة الى تقييم سلامة وصحة أعلاف الفروج من خلال تحديد أنواع فطريات الرشاشيات *Aspergillus spp.* ، وكشف التلوث بالأفلاتوكسينات الكلية في كل من الأعلاف البادئة والنهائية المستخدمة لتغذية الدواجن (الفروج)، حيث تم جمع العينات من ست مزارع مختلفة تابعة لمحافظة طرطوس- سورية، خلال الفترة الممتدة من أيلول 2023 وحتى كانون الثاني 2024، وتمت زراعة العينات في المختبر على مستنبت آجار البطاطا Potato Dextrose Agar (PDA)، كما تم تحليل عينات العلف بحثاً عن محتوى الأفلاتوكسينات الكلية بواسطة جهاز كروماتوغرافيا السائل عالي الأداء HPLC. تم تعريف أربعة أنواع تابعة لفطريات الرشاشيات في عينات الأعلاف المدروسة وهي: فطر الرشاشيات *Aspergillus. terreus* بنسبة (60%)، يليه فطر *Aspergillus .niger* بنسبة (37.1%)، فطر *Aspergillus .flavus* بنسبة (1.9%)، وفطر *Aspergillus .nidulans* بنسبة (1%)، كما تبين أن فطر *A. terreus* كان أبرز الأنواع المعزولة من الأعلاف البادئة بنسبة تردد (96.6%)، في حين كان فطر *A. niger* أبرز الأنواع المعزولة من الأعلاف النهائية بنسبة تردد (78.7%). أظهرت نتائج تقدير الأفلاتوكسينات الكلية من العينات المدروسة أن ثلاثة عينات من الأعلاف البادئة (50%)، وأربعة عينات من الأعلاف النهائية (66.7%) تحتوي على الأفلاتوكسين بمتوسط 0.7 µg/ kg و 1.2 µg/ kg في الأعلاف البادئة والنهائية على التوالي. تستنتج هذه الدراسة أن أعلاف الفروج الجاهزة المدروسة كانت ملوثة بأنواع مختلفة من فطريات الرشاشيات *Aspergillus spp.* وبمستويات منخفضة من الأفلاتوكسينات الكلية، مما يشكل خطراً على صناعة الدواجن بسبب السموم التي تنتجها بعض الأنواع الفطرية، نظراً لأثرها التراكمي.

**الكلمات المفتاحية:** الفروج، إجمالي الأفلاتوكسين، علف بادئ (مهروس)، *Aspergillus*، علف نهائي.