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Original Research Article

Mycotoxins in Poultry Feed in Misan Province, the Southern Part of Iraq

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Abstract: The present study was conducted to detect the contamination levels of some mycotoxin (Afla, Orra, and T2 toxins) in broiler feed by using the ELISA test. Twenty feed samples (500gm/ton for each sample) were collected randomly from broiler flocks feeds which are located in Misan province. The present study was conducted to detect the contamination levels of some mycotoxin (Afla, Orra, and T2 toxins) in broiler feed by using the ELISA test. Twenty feed samples (500gm/ton for each sample) were collected randomly from broilers flock feeds which are located in Misan province. The results revealed aflatoxin contamination in 15 (75%) and only (5) samples were free of aflatoxin. The levels of contamination ranged from 0.1 to 28.5 ppb, and only three (15%) of the aflatoxin-contaminated samples exceeded the US-FDA allowed limits. In terms of Orcha toxins, the findings of the examination revealed that contamination occurred in 7(35%) samples out of a total of 20, while 13 samples were clear of Orcha toxins, with contamination levels ranging from (o.4-34.4) ppb. The levels of ochratoxin in broiler feed did not exceed the European Commission's permissible limits, while T2 toxins were found in 8(40 %) samples and (12) samples were free of T2 toxins, with contamination levels ranging from (3.4-158.3) ppr, and the amount of T2 toxin in (2) samples (10%) exceeded the permissible limits.

Keywords: Mycotoxin, Misan, Fungi, Governorate.

INTRODUCTION

Mycotoxins are natural chemicals produced by fungi that cause toxicity in vertebrates and other animals when introduced in low doses. The molecular weight of these poisons is modest. Aspergillus, Fusarium, PenicilliumAlternaria, and Claviceps spp. are filamentous fungus of the Ascomycota family or molds that produce them (Kagot *et al*., 2019). Mycotoxins are a group of over 400 chemicals, 30 of which have garnered a lot of attention (Alassane *et al*., 2017) and are very important in human and animal health, since they induce acute and chronic disorders (Tola *et al*., 2016, Mousavi *et al*., 2019). Mycotoxin is derived from the Greek words "mykes," which means "fungus," and "oxum," which means "poison" Aflatoxins (AFs), ochratoxins (OTs), and trichothecenes are the most common mycotoxins (TCs). Mycotoxins can't be seen with the naked eye, but they can be seen under ultraviolet light; they also don't have a distinct odor and don't affect the organoleptic features of meals (Winter *et al*., 2019).

Aflatoxins are a group of toxic metabolites produced by a group of common fungi such as Aspergillus flavus and Aspergillus parasiticus, and these fungi produce several types of toxins, including B1, B2, G1, and G2, under ideal environmental conditions of temperature and humidity, and type B1 is one of the most powerful carcinogens. It is the most common contaminant found in chicken feed, particularly in tropical and subtropical areas. Poultry is thought to be one of the most sensitive animals to aflatoxin infection. Furthermore, multiple studies have shown that it can harm the liver and cause cancer in humans. Contamination of various diets with mycotoxins is an issue. feed contamination having negative impacts on animal health, and mycotoxins are frequently detected as natural contaminants in poultry feed raw ingredients, there is a lot of interest in investigating mycotoxins around the world (Khan *et al*., 2011). Aflatoxin has a significant economic influence on the chicken business due to its effects on average weight, growth, immunity, egg output, and other factors. There are four forms of aflatoxins, which are produced by a group of fungi, primarily Penicilliumviridicatum and Aspergillus ochraceus, and are extensively distributed mycotoxins. Orcas come in four different varieties (A, B, C, and D), with type A being the most frequent most toxic. Orcas are thought to be nephrotoxic and nephroncarcinogenic, and a link

has been found between long-term exposure to orcas and morbidity. High quantities of these poisons were discovered in food, blood, and urine samples of persons with urinary tract malignancies in the Balkans (Pittet *et al*., 1998).

Trichothcins, a category of mycotoxins generated by Fusarium acuminatum, Fusarium poae, and Fusarium sporotrichioides (Agag, 2005), are more toxic to poultry than ruminants. T2 toxin's harmful effects are classed as genotoxic and cytotoxic, and it can impact the immune system, digestive system cells, liver, nervous system, and skin, as well as degrade poultry performance (Sokolovic *et al*., 2008). Many countries use fungi in feed on a regular basis to verify that feed and feed ingredients are free of mycotoxins and that products are safe for human and animal health. Because of the aforementioned reasons, the current study used ELISA to determine the degree of contamination of chicken feed with mycotoxins (aflatoxin, T2toxin, and oroca toxins) in Misan Governorate. ELISA is a rapid and accurate method for detecting mycotoxins.

MATERIALS AND METHODS

Sampling Method:

Twenty feed samples (500gm/ton for each sample) were collected randomly from broiler flocks feeds which are located in Misan province. Then all samples were tested by Eliza to determine the degree of contamination with aflatoxin, ochratoxin, and T2 toxins in these feeds. ELISA stands for Enzyme Linked Immuno-Sorbent Assay:

One of the most used methods for determining the quantitative and qualitative levels of mycotoxins. For more than three decades, this approach has been applied in the field of mycotoxins. This method is based on mycotoxin antibodies (Ab), and because the reaction is specialized between the mycotoxin in the sample and the antibody stimulating against it, this feature was exploited in the development of a standard kit for detecting mycotoxins in a variety of foods and agricultural crops. This method is simple, straightforward, quick, accurate, and inexpensive, and it reduces the time required to detect a high number of samples in a short period of time when compared to other methods. This method is also used to estimate mycotoxins quantitatively and qualitatively.

The procedure is divided into multiple parts:

1. Installing the antibodies in an ELISA dish.
2. The toxin-containing sample is added, resulting in a unique interaction between the toxin and the antibody, a complicated component installed at the bottom of the dish's drilling.
3. Using a beaver, thoroughly wash and dig the dish, and then add the enzyme connected to the antibodies.
4. The substance is then added, which interacts with the enzyme and produces a color that varies in concentration according to the concentration of the poison, and the value of the color variation is calculated using the optical absorption device (OD) Optical Density for etching using the ELISA reader, as the color is proportional to the concentration and compared to known concentrations from the standard toxin.

RESULTS

The findings of the current study revealed that aflatoxin contamination was found in 15 out of the 20 tested samples, with a contamination rate of 75%, and three samples (15%) had aflatoxin levels that were above the allowed limits in broiler feed (20ppb). According to US-FDA criteria, the majority of the samples analyzed, 12 samples (60 %), did not exceed the acceptable limits of aflatoxins in broiler feed, and the result also showed only 5 out of 20 tested samples were free of aflatoxins (Table 1).

Table 1: Showed the percentage and evaluation of poultry feed contamination with Aflatoxins

Aflatoxin concentration (ppb)	Number of samples	% percentage
Toxin free	5	25
less than 4	10	50
10-5	1	5
11-20	1	5
more than 20	3	15
Total	**20**	**100**

In terms of Orcha toxins, the findings of the examination revealed that contamination occurred in 7(35%) samples out of a total of 20, while 13 samples were clear of Orcha toxins, with contamination levels ranging from (o.4-34.4) ppb. The levels of ochratoxin in broiler feed did not exceed the European Commission's permissible limits (Table 2).

Table 2: Shows the percentage and evaluation of poultry feed contamination with oocra toxins

Okra toxin concentrate (ppb)	Number of samples	% percentage
Toxin free	13	65
less than 2	6	30
10-5	1	5
more than 10	0	0
Total	**20**	**100**

the results present study found T2 toxins in (8) out of the 20 studied samples, indicating a contamination rate of 40%, and two (10%) of the samples had surpassed the allowed limits in broiler feed 150 ppb according to the instructions of the Iraqi livestock laboratories, the analysis found that 12 (60 percent) of the samples were free of T2 toxins (Table 3).

Table 3: Shows the percentage and evaluation of poultry feed contamination with T2 toxins

T2 Toxin Concentrate (ppb)	Number of samples	% percentage
Toxin free	12	60
less than 25	4	20
150-26	2	10
200-151	2	10
Total	**20**	**100**

DISCUSSION

The current study found that most tested samples of broiler feed were contaminated with aflatoxins at a rate of (75%), which is similar to previous studies in Iraq, where the percentage of aflatoxins contamination in chicken feed was (77.5%) and (92.5%) in the governorates of Najaf and Qadisiyah, respectively.(Malaghi *et al*., 2011; Al-Saeedi, 2014) One of the main reasons for the high levels of aflatoxin is the widespread spread of Aspergillus molds that produce these toxins, as well as their ability to grow in a variety of environmental conditions, particularly at latitudes of 26 and 35 degrees north and south of the equator, and because they are Storagefungi, meaning they can grow on relatively dry crops in a humidity range of 13-18 % (Bennett *et al*., 2003).The high percentage of aflatoxins contamination in the current study could be due to the fact that the samples were gathered and tested during the winter, when humidity levels are high, providing ideal circumstances for the growth of toxins-producing mushrooms. These findings matched those of the researcher (Malaghi *et al*., 2011), who observed a higher percentage of contamination in the winter season compared to the summer, as all samples from the winter season were contaminated with aflatoxins at a rate of (100%) compared to (85.71%) in samples from the summer season, and the reason for this is the difficulty of drying winter crops, particularly yellow maize planted during the fall season. Some researchers in Iraq discovered that the contamination of local maize with mycotoxins was higher than that of imported maize.

Despite the high rate of aflatoxins contamination, 15% of the rations surpassed the permitted aflatoxins in broiler feed limits of ppb (20), as defined by the US-FDA. In countries, there are no set percentages of contamination. For a variety of reasons, including differences in environmental, administrative, geographic, and biological conditions, breed differences, and the nutritional status of chickens consuming these toxins, the world is known for mycotoxins, but (20ppb) is the percentage set by the US-FDA definitions that is applicable in most countries around the world, including Iraq.

A high percentage of aflatoxins has a severe impact on avian performance, resulting in mycotoxication and a high rate of mortality. Although aflatoxins did not exceed the allowed limits in broiler diets in 60% of the samples tested, continual feeding at low levels of aflatoxins may diminish the ability of birds to resist illnesses and cope with stress due to the cumulative action of toxins.

The study's findings revealed the presence of orcatoxin, with a contamination rate of 35%, although the proportion of orca toxins did not surpass the European Commission's permitted limits in broiler diets, which were set at 10 ppb (EFSA, 2009). The findings of this study are identical to those found in the rations of broilers in Al-Qadisiyah and Najaf, which totaled (32%) (Al-Saeedi, 2014). In other countries, where a contamination rate of (100%) was recorded in a survey conducted by researchers in Kuwait, with a contamination range of 4.6-9.6 micrograms/kg feed (Belgin *et al*., 2004), but in a study conducted in northern Italy, despite contamination of all samples with orcas toxins, the concentration of orcas toxins was low, ranging between 0.04-6.5 micrograms/kg feed (Belgin *et al*., (Schiavone *et al*., 2008). Orcatoxin are one of the most dangerous mycotoxins to poultry, and they manifest as a clinical condition as a result of feed contamination in chicken and turkey diets. Despite the presence of orcas in many foodstuffs (such as coffee and dried fruits including raisins and currants), most studies indicate that the highest levels of orcas were found in cereal crops, where these toxins are formed during the period of growth of crops and during Store them under poor storage conditions (Pittet *et al*., 1998).

The results of the study showed the presence of T2 toxins with a contamination rate of (40%) and 2 (10%) samples had exceeded the permissible limits in broiler feed ppb 150, and these results differed from what was recorded in Najaf and Qadisiyah governorates, where the contamination rate reached (86.2%), However, the results of the current study agree with them that 90% of the samples did not exceed the permissible limits of T2 toxins (Al-Saeedi, 2014), and this result may be attributed to the difference in the number of samples examined in the current study, which is 20 samples, while the number The samples examined in the previous study are 130 samples.

CONCLUSIONS

Different mycotoxins were found in broiler feed in Misanprovince at different levels, and the percentage of samples that exceeded the US-FDA permissible limits was low, reaching 15% for aflatoxins and ten percent for T2 toxins, while the percentage of contamination did not exceed the permissible limitsto the Ochratoxin.

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