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Assessment of Aflatoxin contamination of peanut meal, maize and poultry feed mixtures from different agroecological regions in Iraq

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Abstract
Mycotoxins have an impact on poultry production as they are present in feed and have a direct negative impact on poultry performance. Transmission rates of mycotoxins in animal products are generally low (except for aflatoxins in milk and eggs), This results in limited sources of mycotoxins for humans. The risk posed by mycotoxins in human food is much greater. Aflatoxin contamination in chicken feed could be identified as the first assessment of this risk in Iraq. In three agro-ecological zones in Iraq, 201 samples of maize, groundnut meal, broiler and layer feed were collected directly from poultry farms, poultry production sites and poultry feed distributors and analyzed for moisture content and aflatoxin content. The results show that the mean moisture content of maize (14.1%) was significantly (p<0.05) higher than all other commodities (10.0%-12.7%). Approximately 9% of maize samples were positive for aflatoxin, with concentrations overall ranging from <2 to 42 µg/kg. Most of these samples of peanut meal (100%), broiler (93.3%) and layer feeds (83.0%) were positive with concentrations of positive samples ranging from 39 to 950 µg/kg for peanut meal, 2 to 52 µg/kg for broiler feed and 2 to 23 µg/kg for layer feed. The aflatoxin content of layer feed did not vary by agroecological zones, while the highest (16.8 µg/kg) and the lowest (8.2 µg/kg) aflatoxin content of broiler feed were respectively recorded in Babil and in Nagaf agroecological regions. These results suggest that peanut meal may be a high-risk feed and that further research is needed to facilitate the promotion of safe feed for poultry in Iraq.

Keywords: Aflatoxin, feedstuffs, animal feed, broilers, layers, poultry

Introduction
Food plays a vital role in people's lives. Animal protein is one of the most important nutrients for humans. Poultry has been used worldwide for many years as a useful source of protein in the human diet, and animal products, especially meat and poultry liver, play an important role in the human diet. The product is subject to various factors making it unfit for diet for various reasons (Allameh and Razzaghi Abyaneh, 2001) [1]. The discovery of aflatoxin in 1960 killed thousands of turkeys in the UK and continues to pose a threat to the poultry industry and cause significant economic losses due to its sublethal but toxic effects (Allameh and Razzaghi Abyaneh, 2001) [1]. Aflatoxin (AF) is a class of mycotoxins generated by molds. such as A. Flavus, A.parasiticus and A.nominus. Secondary metabolatis of these fungi AF may contaminate the feedstuff especially corn, peanuts and cottonseed. Aflatoxin includes aflatoxin B1, B2, G1, G2, M1, M2 (Sumit R. *et al*., 2010) [24].

Material and Methods
Agroecological Regions
Iraq is divided into three main regions: Dewaniya (I) in the center, Babylon (II) and Najaf in the west (III).
In this study, samples were gathered in three locations chosen based on their relevance in the country's corn and poultry production.
Sampling “From February 2023 to July 2023, 201 samples of animal feed and poultry feed were randomly selected from small chicken farms and poultry feed (30 samples of broiler feed, 41 samples of peanut meal, 53 samples of laying hen feed and 77 samples of corn) production bases or poultry feed distributors in the above three regions.

Collect groundnut meal and poultry feed in Najaf, Babylon and Dewaniya. These areas were chosen because they have the highest concentration (95%) of chicken farms in Iraq. Maize samples (40 yellow maize samples and 37 white maize samples) were collected in the Dewania and Babil districts during the same period. These were chosen because of their importance to corn cultivation. The samples were chosen because of their importance in corn production. Samples were stored in plastic bags at room temperature (20-25 °C) until analysis in December 2019; all samples were sealed under vacuum to prevent air exchange between the samples and the storage environment.

Moisture Content Determination:

The moisture content of samples was tested using the conventional oven technique (AOAC, 1999) [4]. The samples were weighed, dried in triplicate at 100 °C to constant weight, and the mean moisture content on a % dry basis computed.

Aflatoxin Content Determination

Aflatoxin Extraction

All of the samples were crushed with a Romer Mill (Romer series II® MILL), and 5.0 g of each was weighed and mixed with 0.5 g sodium chloride. In a controlled environment shaker, 10 mL of 80% methanol solution (methanol: water, 80:20 v/v) was introduced and shaken at 225 rpm for 4 minutes at 25 °C (New Brunswick CO. INC, EDISON, N.J., USA). The mixture was filtered using fluted filter paper (Folder Grade: 1289, VICAM, A Waters Business), and 2 mL of the filtrate was diluted with 8 mL of distilled water in a clean tube before being combined for 2 minutes on a Denley Spiramix linear mixer (Denley, Sussex, UK). Two milliliters of diluted filtrate (0.2 g sample equivalent) were run through the apparatus an Aflatest®-P affinity column at a rate of 1 to 2 drops/second, and the column was then washed twice at the same rate with 5 mL of distilled water. The aflatoxin material coupled to the affinity column was eluted with 1.0 mL HPLC grade methanol at 1 to 2 drops/second and collected in a glass tube.

Aflatoxin Quantification

One mL of Aflatest® developer solution was added to the Aflatest-P column eluate, mixed, and total aflatoxin (B1 + B2 + G1 + G2) concentrations (g/kg) were detected after 60 seconds using a Vicam fluorometer (Series-4EX, Source Scientific LLC, USA) calibrated with a methanol blank, according to the standard manufacturer's protocol. This method's detection limit was 2.0 g/kg, while its upper limit was 300 g/kg. Extracts were diluted an additional X5 for samples containing more than 300 g/kg, resulting in a maximum concentration of 1500 g/kg.

Data Analysis: Data were summarized and analyzed using SPSS (version 12.0), and Duncan's multiple range was performed to assess variations in averages across samples collected from different areas (P = 0.05).

Result

Moisture content

Moisture content is a risk factor for aflatoxin buildup in maize, groundnuts, and other crops after harvest. Maize had a greater mean moisture content (P 0.05) than all other commodities (10.0%-12.7%), with peanut meal having the lowest (10.0%). (Table 1). Maize moisture content varied from 11.7% to 17.0% with an average of 13.7% in Babil and 12.9% to 17.7% with an average of 15.4% in Dewania. In the Babil and Nagaf areas, the average moisture content of peanut meal was 10.1 and 9.8%, respectively. The extremes of moisture content in chicken feeds were reported in Nagaf, with an average of 10.7% in broiler feed and 12.7% in layer feed.

Table 1: shows the moisture content of maize, peanut meal, and chicken feed combinations in Iraq's various agroecological areas.

Commodities	Agroecological zone	N	Range (%)	Mean withen the agro-zone (%)	Mean	SEM
Mazie (n=77)	Babil	62	11.7-17.0	13.7	14.1^{a}	0.8
	Dewania	15	12.9-17.7	15.4		
Peanut meal (n=41)	Babil	33	8.5-11.8	10.1	10.0^{d}	0.1
	Nagaf	8	9.1-10.5	9.8		
Broilers feed (n=30)	Babil	13	9.9-14.7	12.2	11.3^{c}	0.3
	Nagaf	17	8.4-17.0	10.7		
Layers feed (n=53)	Babil	30	10.2-16.1	12.6	12.7^{b}	0.2
	Nagaf	20	10.8-15.3	12.7		

Aflatoxin Contamination

The levels of aflatoxin in the four feed groups were measured. Aflatoxin levels in all peanut meals above the method's detection limit (2 g/kg). Aflatoxin values varied from 2 to 42 g/kg in 9.1% (7/77) of maize. Overall, 57.1% (4/7) of positive maize samples had less than 5 g/kg of aflatoxin, whereas 42.9% (3/7) had more than 5 g/kg. Broiler feeds (11.2 g/kg) had about twice as much aflatoxin as layer feeds (6.6 g/kg). Similarly, 35.7% (10/28) of broiler feed samples had more above 10 g/kg of aflatoxin, while 22.7% (10/44) of layer feeds did not (Table 2). Aflatoxin was detected in 87% of broiler and layer feeds (72/83).

Table 2: Shows the levels of aflatoxin in maize, peanut meal, and chicken feed combinations in Iraq.

Combinations	Aflatoxin occurence	Range (mg/kg) (min-max)	Mean(mg/kg)	SEM	Frequency (of positive)
Mazin (n=77)	7/77(9.1%)	≤2-42mg/kg	1.0^{a}	0.5	<5mg/kg 4/7(57.1%) >5mg/kg 3/7(42.9%)
Peanut meal (n=41)	41/41(100%)	39-950mg/kg	161.4^{b}	27.5	<5mg/kg 4/41(9.8%) 50-100mg/kg 21/41(51.2%) >100mg/kg (16/41(39.0) <5mg/kg (10/28.6%)
Broiler feeds (n=30)	28/30(93.3%)	≤2-52mg/kg	11.1^{a}	2.2	5-10mg/kg 10/28 (35.7%) >10mg/kg 10/28(35.7%) <5mg/kg 15/44(34.1%)
Layer feeds (n=53)	44/53(83.0%)	≤2-23mg/kg	6.6^{a}	0.7	5-10mg/kg 19/44(43%) >10mglkg 10/44(22.7)

The mean aflatoxin level in Nagaf peanut meal (224 g/kg) was greater than in Babil (146 g/kg); all samples tested positive for aflatoxin (i.e., 100% occurrence) (Figure1b). Positive maize from Dewania had an average total aflatoxin content of 2.40 g/kg, whereas positive samples from Babil had an average total aflatoxin content of 11.88 g/kg (Figure 1a); averages for all samples were 1.35 g/kg and 0.16 g/kg for Babil and Dewania, respectively. Aflatoxin levels in maize were 10% (6/62) and 7% (1/15) in Babil and Dewania, respectively. Broiler feed occurrence was 92% (12/13) in Babil and 94% (16/17) in Nagaf. The presence of Babil in layer feeds was 85% (28/33) and Nagaf was 80% (16/20).

The overall aflatoxin concentration of layer feeds was consistent, averaging 7.9 g/kg for both Nagaf and Babil (Figure 2). However, in broiler diets, Nagaf had the lowest aflatoxin concentration (8.2 g/kg) compared to Babil (16.9 g/kg). For all commodities, the connection between agrecological zones and overall aflatoxins concentration was not significant.

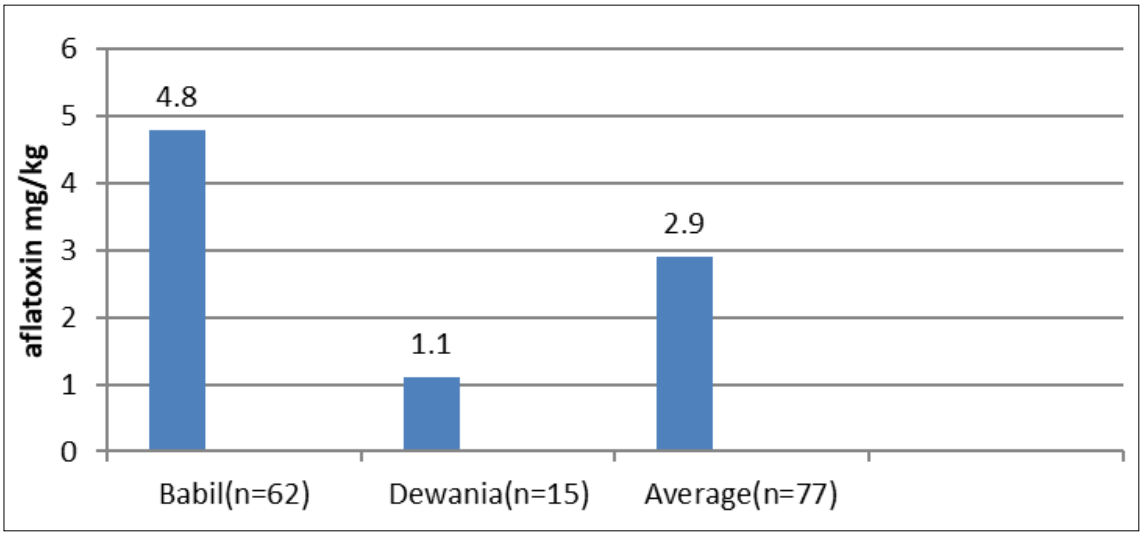

Fig 1a: Average aflatoxin content of aflatoxin positive maize samples.

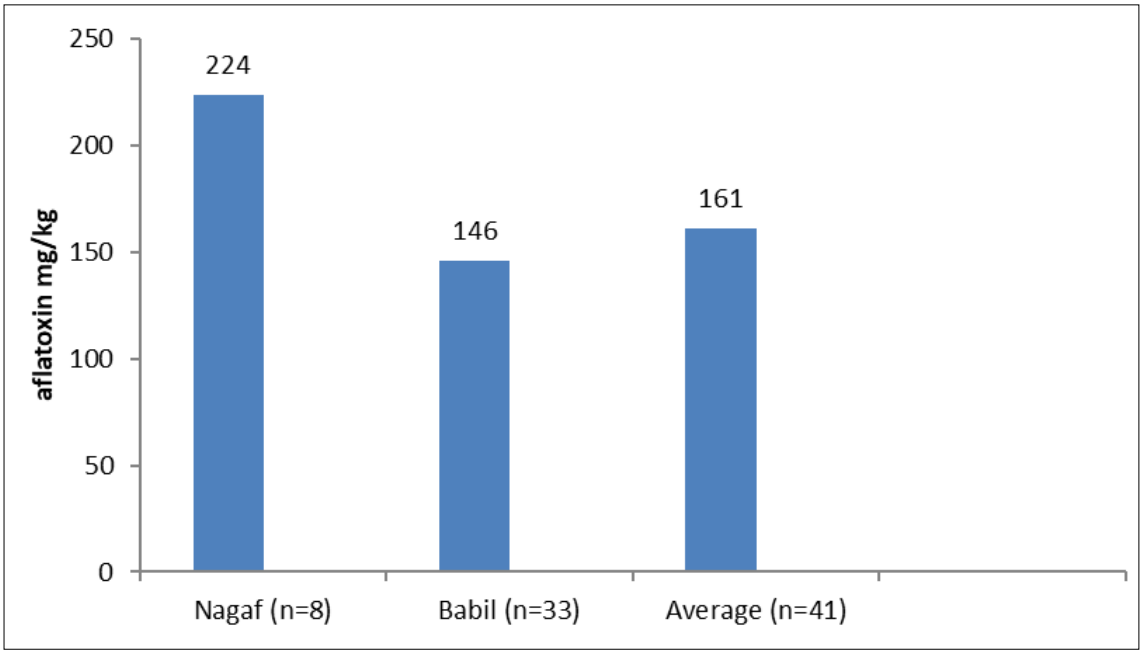

Fig 1b: Average aflatoxin content of aflatoxin positive peanut meal samples.

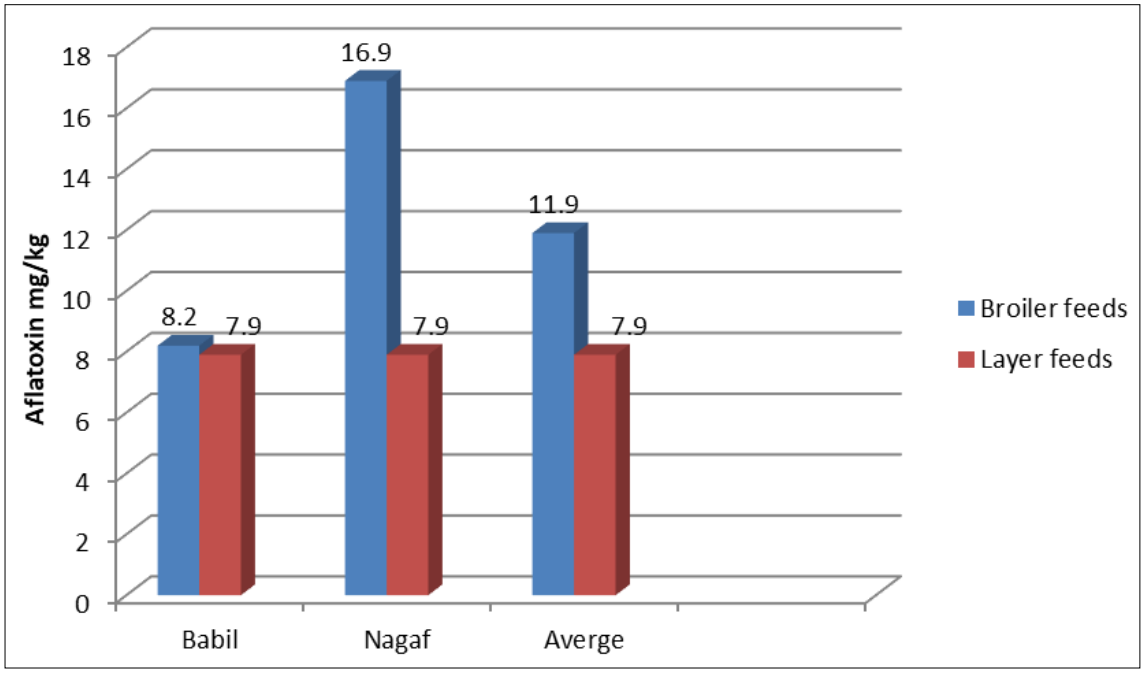

Fig 2: Average aflatoxin content of poultry feed mixtures collected in two agroecological regions in Iraq.

Discussion

The Iraqi poultry industry produces 500,000 day-old chickens per week. Total animal feed production capacity, including poultry feed, is expected to exceed 140,000 tonnes per year, compared to less than 100,000 tonnes currently due to the ban on chicken imports (Pouomogne, V. 2007) [27]. Mycotoxins such as aflatoxin can negatively affect chicken productivity and may be a hazard to human health. The study examined moisture content, a key factor in aflatoxin formation, and aflatoxin levels in various chicken feeds from poultry farms and three Iraqi provinces with the largest corn production. Accumulation of aflatoxins was significant in meals, especially peanut flour. The aflatoxin content in peanut meal exceeded the allowable limit of 20 g/kg (up to 950 g/kg) recommended by the U.S. Food and Drug Administration's Aflatoxin Agency (FDA) for chicken feed formulations. In this case, these chemicals pose a risk to the Iraqi poultry industry and its customers.

"Favorable temperature and water activity, which is an inherent characteristic for moisture content, are critical for the growth of mycotoxigenic fungus and the generation of mycotoxin. In general, severe temperatures and drought in relevant areas may render crops more susceptible to aflatoxins, as shown in Kenya in recent years 16. (FAO. 2000) [10]. Overall, climatic conditions in the tropical region are favorable for fungal development, with high relative humidity (Marasas, W.F.O, 2001) [16] (Hell, K. *et al*., 2003) [13], high temperature and moisture content (Velluti, A.; *et al*, 2000) [26] (Peterson, S.W. *et al*., 2001) [20] and limited aeration (Fandohan, P.; *et al*, 2006) [9]. The aforementioned climatic and environmental circumstances are ideal for the proliferation of fungus, particularly those belonging to the genera Aspergillus, Penicillium, and Rhizopus, which generate and release spores (Domsch, K.H. *et al*., 1980) [8]. Maize moisture content varied from 11.7% to 17.7%, with a mean of 14.1%. (Table 1). This is congruent with the findings of (Kaaya, A.N.; Kyamuhangire, 2006) [13], who observed a mean moisture content of maize of roughly 13% in three Iraqi Agroecological areas. Moisture content of 15% is within the safe storage range for maize, according to (Magan, N.; Lacey, J, 1988) [16]. Molds, on the other hand, have been observed to develop at temperatures ranging from 10 to 40 °C and at relative humidity levels more than 70% (Smith, J.E. 1997) [23] (Lanyasunya, T.P. *et al*., 2005) [15]. Some fungi have even been shown to be capable of growing on a dry surface and on feeds containing 12%-13% moisture by (Lacey, J. 1991) [14] and (Thomson, C.; Henke, S.E. 2000) [25]. The moisture content of maize (14.1%), broiler feeds (11.3%), and layer feeds (12.7%) in our study falls within this range and might be acceptable for fungal growth and toxin generation if the pH and relative humidity conditions are good. When compared to Nagaf, the moisture level of broiler feeds collected at Babil was higher. This might be explained by Babil's hot climate and high relative humidity (>80%) in comparison to Nagaf's circumstances (Ngoko, Z.; *et al* 2001) [16].

This study discovered that aflatoxin contaminated roughly 9% of the maize used in chicken feed formulations in Iraq, with concentrations ranging from 2 to 42 g/kg, with an average of 1.0 g/kg (Table 2). This average was lower than the results of (Rodrigues, I. 2011) [21]. Our findings contradict with those of (Kaaya, A.N.; Kyamuhangire, W. 2006) [13], who found 88%, 78%, and 69% of maize infected by aflatoxin with average concentrations of 30, 22, and 12.8 g/kg in Uganda's Mid-Altitude damp, Mid-Altitude dry, and Highland zones. The aflatoxin concentration of peanut meal varied from 39 to 950 g/kg in the current research, with an average of 161.4 g/kg (Table 2). These findings are lower than those of (Abdelhamid, A.M. 1990) [1], who found an average aflatoxin content of 400 g/kg in Egyptian peanut meal.

The majority of chicken feeds (87%) tested positive for aflatoxin, and broiler feeds contained twice as much aflatoxin as layer feeds (6.6 g/kg), but the difference was not statistically significant. The reason for the higher aflatoxin content in broiler feed may be that broiler chickens require higher protein than laying hens. To meet this demand, farmers use large amounts (up to 20%) of peanut flour in their formulations because it is the cheapest source of protein and more susceptible to aflatoxin contamination. The prevalence of aflatoxins in chicken feed is currently quite high (93% and 83% in broiler feed and layer feed, respectively), while the incidence of aflatoxin in positively tested poultry feed was 24% and 26%, with an average of Concentrations of 7 g and 2

g/kg were reported in North America and South America in 2009 and 2011, respectively. (Rodrigues, I.; Naehrer, K. 2012) [12].

The study found that the concentrations of aflatoxins in broiler feed were highest in the Babylonian region and lowest in the Najaf agro-ecological region. Environmental conditions in Babylon, especially temperature and relative humidity, may have contributed to this development. The fact that the majority of chicken feed from the Babylonian region (60% of Iraqi poultry production) tested positive for aflatoxin suggests that poultry production may be affected (Bryden, W.L. 2012) [6], poultry meat and eggs may be affected. The result is aflatoxin contamination (Oliveira, C.A.F. *et al*., 2000) [19] and (Bintvihok, A. *et al*., 2002) [5].

Conclusion

Broiler feed had the highest aflatoxin content, and laying hen feed had the same aflatoxin content. In particular, high temperatures and relative humidity/humidity promote fungal growth and mycotoxin formation. Peanut meal was the preferred substrate for fungal growth and had the highest level of contamination. Insufficient storage conditions may lead to fungal growth and aflatoxin formation in Iraqi feed and poultry feed. There is an urgent need to find a practical and cost-effective anti-mold and detoxification technology for aflatoxin-containing chicken feed.

Preventative methods against fungal toxicity

The prevention approach starts with growing crops, selecting for resistant mushrooms and regularly rotating crops, especially yellow corn, which makes up the bulk of chicken feed. In terms of preservation, these products are stored in warehouses with proper storage conditions and protected from direct sunlight. Due to the possible rapid growth of the fungus, when preparing ready-to-eat feeds, care must be taken that only a few days are required to prepare the mixed volume, and the addition of anti-mycotoxins to the feed is considered one of the most important preventive measures that must be observed in feeding poultry. Precautions are summarized below:

1. Feed supplies are stored in warehouses that meet the necessary conditions, such as temperature, humidity, and ventilation.
2. Feed silos should not be in direct sunlight.
3. Keeping enough feed on hand to last a few days.
4. Washing and sanitation of feeders and drinkers in wards and silos on a regular basis.
5. Add anti-mycotoxins to bird drinking water and allow them to drink every 10 days with a powerful anti-toxin that removes all types of toxins.

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