

## SITUATION OF AFLATOXIN RESIDUES IN CHICKEN AND DUCK MEAT

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### ABSTRACT

Species of *Aspergillus* produce a class of mycotoxins known as aflatoxins. For both humans and animals, aflatoxins are poisonous and cancer-causing agents. A potential threat to consumer health, therefore, exists when aflatoxins are found in food. In market meat, particularly in chicken and duck, there have been few reports of aflatoxins residues. As a key source of protein, poultry products are one of the foods that are consumed widely in Egypt. So, the goal of this study was to use HPLC for measuring the total aflatoxins (AF) and aflatoxin B1, B2, G1 and G2 levels in chicken and duck flesh and liver. A total of 100 samples were collected throughout the study period, including 50 samples of chicken meat (25 muscles, 25 liver), and 50 samples of duck meat (25 muscles, 25 liver). Findings showed that the samples investigated varied significantly (p values). The highest total aflatoxin residues were detected in the liver in both chicken and duck ( $6.61 \pm 1.51$ ,  $6.15 \pm 1.75$  ppb respectively), while in the muscles it was lower ( $0.07 \pm 0.005$ ,  $0.071 \pm 0.03$  ppb respectively). Moreover, the amount of aflatoxin residue found was highest in duck liver ( $6.15 \pm 1.75$  ppb) 80% from total examined samples, whereas it was significantly lower in chicken (Liver, muscles) and duck muscles. The liver of the examined chicken and duck samples showed a significant differential variation (p values), however the muscle variation was statistically negligible. The presence of contamination in every sample is a health hazard, and it appears that more research is needed.

**Keywords:** Aflatoxin, Chicken, Duck, Muscles, Liver.

### INTRODUCTION

Mycotoxins are byproducts of filamentous fungi or mould that are found in the soil, grain, forage, and silage. While not necessary

for fungal growth and reproduction, they can cause biochemical, physiological, and pathological alterations in a variety of species (Wang *et al.*, 2018). Aflatoxin A, ochratoxin A, T-2 toxin, nivalenol, zearalenone, and Deoxynivalenol are the most prevalent mycotoxins' components (Pitt and Hocking, 2022). The most hazardous of these are aflatoxins, which are secondary metabolites formed from polyketides produced by fungi such as *Aspergillus*

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*flavus*, *A. parasiticus*, and *A. nomius* (Kumar *et al.*, 2017; Shabeer *et al.*, 2022). There are currently around 20 known aflatoxin compounds, however, the difurocoumarocyclopentenone group (aflatoxin B1 and B2) and the difurocoumarolactone group are the most major ones in terms of food and feed safety (aflatoxin G1 and aflatoxin G2) (Shabeer *et al.*, 2022). Aflatoxin contamination is one of the most serious problems nowadays since it can have a considerable impact on the food chain. At the top of the food chain, humans frequently eat contaminated meals that might be either plant- or animal-based. Food insecurity brought on by aflatoxins contamination can have negative social, political, and economic effects on humanity in addition to adverse effects on human health (Pickova *et al.*, 2021).

Poultry is a significant source of protein and other nutrients for human nutrition, such as chicken and duck (Bordoni and Danesi, 2017). Around 33% of the world's meat consumption is accounted for by the poultry industry, which is predicted to rise at a rate of 2-4% per year (Sebho, 2015; Tatfo Keutchatang *et al.*, 2022). The majority of agricultural items used to make poultry feed, including maize, groundnuts, and wheat, have the potential to be contaminated with mycotoxins, particularly aflatoxins (Moretti *et al.*, 2017; Ráduly *et al.*, 2020). In chicken feeds, aflatoxins have been found anywhere between 64% and 100% of the time (Mokubedi *et al.*, 2019; Aboagye-Nuamah *et al.*, 2021; Ochieng *et al.*, 2021). It was noted that the liver and muscle tissues of chickens contain the most aflatoxins residues, as opposed to any other organs (Darwish *et al.*, 2016; Faten *et al.*, 2016). This emphasizes how crucial it is to keep an eye on aflatoxins in processed broilers to ensure the protection of public health, it was crucial to ascertain the extent of these pollutants in poultry and duck meat, as well as to research the extent of their impact on human health, the economic viability of these fungal pollutants, and how to prevent them with

recommendations related to food safety to improve consumer protection.

## MATERIALS AND METHODS

### 1. Chemicals and reagents

Sigma (St. Louis, Missouri, USA) provided standards for total aflatoxins that were purified to 99%; standard stock solutions were made in acetonitrile using the Association of Official Analytical Chemists (AOAC) technique (IARC, 2002). A workable solution made in acetonitrile that was kept for a year at -20 °C in amber glass vials. Solid phase extraction cyanopropyl cartridges (SPE-CN), which were acquired from Varian, were used to clean the samples. Si-column from Thermo was used for the HPLC analysis. Tedia was used to get high-grade methanol, chloroform, acetonitrile, ethyl acetate, acetic acid, formic acid, toluene, and HPLC water (Fairfield, OH, USA). Sigma (St. Louis, Missouri, USA) provided the diatomaceous earth (SiO<sub>2</sub>), and the American Chemical Society provided the sodium sulphate anhydrous (99.5%) utilized in the study (ACS, Madrid, Spain).

### 2. Samples collection and processing

In Beheira Governorate of Egypt, 20 processed chicken meat specimens (muscle and liver; n = 25 for each type) and 20 processed duck meat specimens (muscle and liver; n = 25 for each type) were chosen at random from licensed retail markets that had attained good hygiene practice (GHP). The collected samples were tagged, put into sterile polyethylene bags, and quickly transported to the Microbiology lab at the Animal Health Research Institute in Damanhur under strict aseptic conditions in an icebox.

### 3. HPLC Determination

Twenty microliters of the mixture, together with an isocratic mobile phase made of deionized water, were injected into the HPLC: Methanol: Acetonitrile (60:20:20 v/v/v) using a gradient method with a flow rate of 1 ml/min at a temperature of 30°C. A

reversed-phase column (Extend-C18, Zorbax column, 4.6 mm 250 mm, 5 m, Agilent Co.) was used for the separation. A fluorescence detector with wave lengths of 360 nm excitation and 440 nm emission was used to do the detection. The area under the curves, which was automatically extrapolated using ChemStation software, was used to extract and compute the quantity of residues in the samples. Aflatoxin mixture standards with concentrations of 275, 550, 1375, 2750, and

4450 ppb were used to create a calibration curve.

#### 4. Statistical analysis

The standard deviation of the mean (SD) for each group was used to express variability in the data, which were given as means. According to Feldman *et al.* (2003), the statistical evaluation of the results was done using the t-test. By mean values that displayed significant differences  $P < 0.05$ , the importance was assessed.

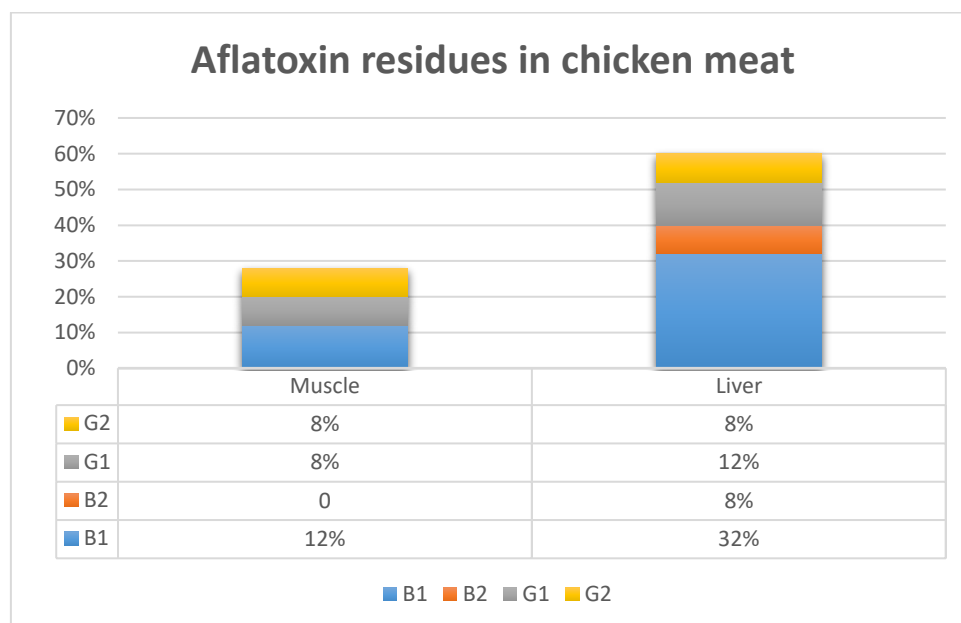
## RESULTS

**Table 1:** Results of aflatoxin residues means in PPb of chicken muscle.

Aflatoxins	Chicken Muscles (25 samples)				
	European permissible limits (EC) 4 ppb		Egyptian permissible limits (EOS) 10 ppb		American permissible limits (FDA) 20 ppb
	No +ve samples	%	Min	Max	Mean± standard deviation
B1	3	12%	0.01	0.02	0.04±0.005
B2	0	--	--	--	--
G1	2	8%	0.01	0.02	0.015± 0.005
G2	2	8%	0.01	0.02	0.015± 0.005
Total	7	28%	0.01	0.05	0.07±0.005

**Table 2:** Results of aflatoxin residues means in PPb of chicken livers.

Aflatoxins	Chicken livers (25 samples)				
	European permissible limits (EC) 4 ppb		Egyptian permissible limits (EOS) 10 ppb		American permissible limits (FDA) 20 ppb
	No +ve samples	%	Min	Max	Mean± standard deviation
B1	8	32%	2	3	2.63±0.484
B2	2	8%	0.1	0.2	0.15± 0.05
G1	3	12%	2	3	2.33±0.471
G2	2	8%	1	2	1.5± 0.5
Total	15	60%	4	8.1	6.61± 1.51



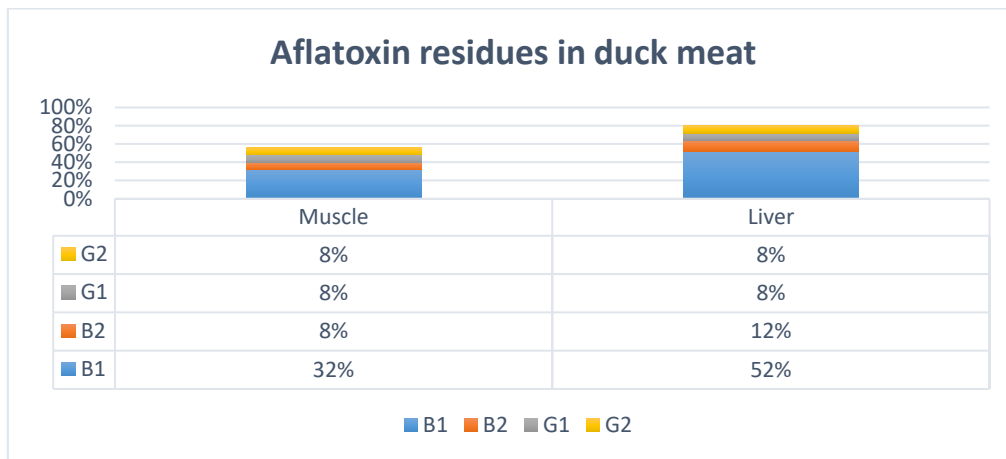
**Figure 1:** Aflatoxin residues percentage in PPb of chicken muscle and liver.

**Table 3:** Results of aflatoxin residues in PPb of duck muscle.

Aflatoxins	Duck Muscles (25 samples)				
	European permissible limits (EC) 4 ppb		Egyptian permissible limits (EOS) 10 ppb		American permissible limits (FDA) 20 ppb
	No +ve samples	%	Min	Max	Mean± standard deviation
B1	8	32%	0.01	0.05	0.026±0.015
B2	2	8%	0.01	0.02	0.015±0.005
G1	2	8%	0.01	0.02	0.015±0.005
G2	2	8%	0.01	0.02	0.015±0.005
Total	14	56%	0.02	0.09	0.071±0.03

**Table 4:** Results of aflatoxin residues in PPb of duck liver.

Aflatoxins	Duck Livers (25 samples)				
	European permissible limits (EC) 4 ppb		Egyptian permissible limits (EOS) 10 ppb		American permissible limits (FDA) 20 ppb
	No +ve samples	%	Min	Max	Mean± standard deviation
B1	13	52%	2	3	2.32±0.778
B2	3	12%	1	2	1.33±0.471
G1	2	8%	1	2	1.5±0.5
G2	2	8%	1	1	1.0 ±0.0
Total	20	80%	3.2	8	6.15±1.75



**Figure 2:** Aflatoxin residues percentage in PPb of duck muscle and liver.

## DISCUSSION

Mycotoxins are known to cause human and animal fatalities and illnesses, veterinary and medical costs, decreased productivity, loss of livelihoods, food and feed losses, and contamination, all of which have a detrimental economic and societal impact (Atherstone *et al.*, 2016; Puri *et al.*, 2019; Pleadin *et al.*, 2021; Marrez and Ayesh, 2022). Our study involves detecting the residues of aflatoxins types "B1, B2, G1, G2 and total aflatoxins residues" in chicken and ducks due to the toxicological and carcinogenic influence of aflatoxins residues on human health and the lack of studies of aflatoxins residues in chickens.

### 1. Aflatoxin residues in chicken

Aflatoxins residues by using HPLC showed that the mean of total aflatoxins residues in chicken muscle meat was  $0.07 \pm 0.005$  ppb. There are 7/25 (28%) were positive for aflatoxins with a predominance of AFB1 3/25 (12%). The mean AFB1 was  $0.04 \pm 0.005$  ppb. Maximum residues in all samples were 0.02 and the minimum was 0.01 ppb. While the mean value of AFG1 and AFG2 was  $0.015 \pm 0.005$  ppb in both types of aflatoxins. On the other hand, the mean of total aflatoxins residues in chicken livers was  $6.61 \pm 1.51$  ppb. There are 15/25 (60%) were positive for aflatoxins with a predominance of AFB1 8/25 (32%). The mean AFB1 was  $2.63 \pm 0.484$  ppb. Maximum residues in all samples were 3 ppb and the

minimum was 2 ppb. While the mean value of AFG1 and AFG2 was  $2.33 \pm 0.471$  ppb and  $1.5 \pm 0.5$  ppb respectively (Table 1, 2). There was a significant difference between aflatoxin residues in chicken muscle meat and liver ( $P < 0.0001$ ). The current findings in Tables 1, 2 are consistent with those of Śliżewska *et al.* (2019) who said that although the liver is the main site for aflatoxin residues, they can also be discovered in muscles, stomach, kidneys, adipose tissue, and meat. The findings were consistent with those made by Herzallah (2013) who claimed that the liver had the greatest levels of both AFB1 and all other aflatoxins. The current findings are in stark contrast to those made by Abo El-Yazeed *et al.* (2015) who recorded that the residual amounts were higher in the muscles than in the liver. Regarding the present study results, the mean values of detected aflatoxins in the examined samples were lower than the maximum permissible limit recommended according to Egyptian Standard (EOS, 2003) 10 ppb and United States Food and Drug Administration (FDA, 1999) (20ppb in food for human conception.) and European Union (EU) (EC, 2007, 2010) permissible limits of AFB1 and total aflatoxin (2 and 4  $\mu\text{g}/\text{kg}$ ) with high differences. This presents the seriousness of the probability incidence of the above-mentioned risks caused by the intake of contaminated edible chicken offals with aflatoxins. Food and drug administration (FDA, 1999) Stated that aflatoxins

especially B1, B2 and G1 were the most common toxin found in human food stuffs its health effect include acute toxicity and impaired mental development, especially for children, old age persons, and pregnant and lactating women. Due to the public health risk of aflatoxins residues and their presence in our food threatening human health, our results agreed with those reported by Bennett and Klich (2003) and Miliță *et al.* (2010) who revealed that the liver is the target organ for aflatoxins. In human aflatoxins residues are lethal due to intoxication, while in chronic accumulation aflatoxins poisoning lead to hepatocellular carcinoma. aflatoxins (AFs), which are a group of heterocyclic metabolites produced by the fungi of the genus *Aspergillus*, particularly *Aspergillus flavus* and *Aspergillus parasiticus* that frequently contaminate animal feed and human food, causing illness and death to consumers (Magnussen and Parsi, 2013).

## 2. Aflatoxin residues in ducks

Aflatoxins residues by using HPLC showed that the mean of total aflatoxins residues in duck muscles meat was  $0.071 \pm 0.03$  ppb. There are 14/25 (56%) were positive for aflatoxins with a predominance of AFB1 8/25 (32%). With a mean value  $0.26 \pm 0.015$  ppb, the maximum residues in all samples were 0.05 and the minimum was 0.01 ppb. While the mean value of AFG1 and AFG2 was  $0.015 \pm 0.005$  ppb. 2/25 (8%) were positive in both types of aflatoxins. On the other hand, the mean of total aflatoxins residues in duck livers was  $6.15 \pm 1.75$  ppb. There are 20/25 (80%) were positive for aflatoxins with a predominance of AFB1 13/25 (52%) the maximum residues in all examined samples were 3 ppb and the minimum was 2 ppb. from. The mean AFB1, AFB2, AFG1 and AFG2 were ( $2.32 \pm 0.778$ ,  $1.33 \pm 0.471$ ,  $1.5 \pm 0.5$  and  $1.0 \pm 0.0$  ppb respectively) (Table 3, 4). There was a significant difference between aflatoxin residues in duck muscles meat and liver ( $P < 0.0001$ ). The current findings in Table 4 are consistent with those made by Awad *et al.* (2019) who stated that although aflatoxins

residues can be discovered in the liver, muscles, stomach, kidneys, adipose tissue, and meat, the liver serves as a harbor for these residues. In the same line, the findings corroborated those of Darwish *et al.* (2016) who claimed that the liver had the highest levels of total and AFB1 aflatoxins. The current findings are in stark contrast to those made by Abo El-Yazeed *et al.* (2015) who discovered that the residual amounts were higher in the muscles than in the liver. Our results in this study show that the mean values of detected aflatoxins in the examined samples of ducks were lower than the maximum permissible limit recommended according to Egyptian Standard (EOS, 2003) 10 ppb and United States Food and Drug Administration (FDA, 1999) that established regulatory working guidelines on the acceptable levels of aflatoxins in human foods set at 20 ppb for total aflatoxins, with the exception of milk which has an action level of 0.5 ppb of aflatoxins (Bullerman, 1979). At the same time, the mean values of detected aflatoxins in the examined samples were lower than the maximum permissible limit recommended by European Union (EU) (EC, 2007, 2010) permissible limits of AFB1 and total aflatoxin (2 and 4  $\mu\text{g}/\text{kg}$ ) with high differences but it should be noted that the production of aflatoxins may be accelerated by improper production and handling of foods. Human exposure to mycotoxins occurs frequently due to the consumption of mould-contaminated agriculture products or the transition from feed to poultry meat (Wafia and Hassan, 2000) There are four naturally occurring AFs: aflatoxin B1, B2, G1 and G2, and all of them are toxic, mutagenic and carcinogenic compounds specially for Children's, old age persons, pregnant and lactating women (CAST, 2003) having been classified by the International Agency for Research on Cancer as belonging to group 1 (substances that are carcinogenic for humans) (IARC, 1993). A potential immune-suppressant and nutritional interference effect has also been reported (Williams *et al.*, 2004), as have mutagenic, teratogenic

and hepatotoxic effects (Kensler *et al.*, 2011).

### 3. Aflatoxin residues variation in chicken and ducks

Due to their unique mycotoxin metabolism compared to other species, ducks are around 200 times more susceptible than chickens. In comparison to broilers, ducks have a higher bio-activation activity of aflatoxin and a lower rate of detoxification and removal of this mycotoxin (Diaz and Gonzalo, 2011). The results of the present study revealed a statically insignificant variation in the level of total aflatoxins and AFB1 in the muscles of both chicken and duck. On the other hand, there was a significant elevation of total aflatoxins and AFB1 in the liver of ducks than in chicken ( $P = 0.0045$ ). Aflatoxin may be introduced to poultry meat through the use of contaminated additives and spices which used to the poultry meat quality (El-Bouhyetal. 1994). It is of great magnitude to mention that aflatoxinB1 is the most potent carcinogenic even at very low concentrations as compared with other types of aflatoxins (WHO 2002).

### CONCLUSION

In conclusion, the total aflatoxin residues were higher in the liver than muscles. The ducks are more susceptible than chickens for aflatoxins especially, AFB1. Contamination of duck liver with aflatoxins leads to a public health risk threatening human beings because AFB1 is an extremely potent carcinogenic effect. Chicken and duck muscles and livers in this study were subjected to various degrees of contamination through meat processing. Therefore, a concerted effort should be made to maintain sanitary conditions in processing, preparation and handling. This can be controlled by the application of strict hygienic measures during slaughtering, struggling as well as efficient bleeding should be considered. All meat and establishments develop and implement a system of preventive control designed to

improve the safety of their products, known as Hazard Analysis and Critical Control Points (HACCP).

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## وضع بقايا السموم الفطرية في لحوم الدجاج والبط

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تنتج أنواع الفطريات فئة من السموم الفطرية تعرف باسم الأفلاتوكسينات. بالنسبة لكل من البشر والحيوانات، تعتبر الأفلاتوكسينات سامة ومسببة للسرطان. لذلك يوجد تهديد محتمل لصحة المستهلك عند العثور على الأفلاتوكسين في الغذاء. في لحوم السوق وخاصة في الدجاج والبط، توجد تقارير وأبحاث قليلة عن بقايا الأفلاتوكسينات في لحوم الدجاج والبط والتي تعتبر المصدر الرئيسي للبروتين للانسان، تعد منتجات الدواجن واحدة من الأطعمة التي يتم استهلاكها على نطاق واسع في جميع أنحاء مصر. لذلك، كان الهدف من هذه الدراسة هو استخدام جهاز الكروماتوجرافي عالي الكفاءة (HPLC) لقياس إجمالي مستويات الأفلاتوكسين (AF) والأفلاتوكسين (AFB1, B2, G1 and G2) في لحم الدجاج والبط ، بما في ذلك العضلات والكبد. تم جمع عدد 100 عينة طوال فترة الدراسة ، تشمل عدد 50 عينة من لحوم الدجاج (25 عضلة ، 25 كبد)، و 50 عينة من لحم البط (25 عضلة ، 25 كبد). أظهرت النتائج أن الكبد والعضلات في عينات الدجاج والبط قيد الدراسة تختلف معنوياً. وتم الكشف عن أعلى مجموع بقايا الأفلاتوكسين في الكبد في كل من الدجاج والبط (6.11 ± 1.75 ، 1.51 ± 6.15 ، 1.75 ± 6.15 جزء في البليون على التوالي)، بينما كانت أقل في العضلات (0.07 ± 0.005 ، 0.071 ± 0.03 جزء في البليون على التوالي). علاوة على ذلك ، كان متوسط كمية بقايا الأفلاتوكسين الموجودة أعلى في أكباد البط 6.15 ± 1.75 جزء في البليون بنسبة 80% من إجمالي العينات المفحوصة ، بينما كانت أقل بشكل ملحوظ في الدجاج (الكبد والعضلات) وعضلات البط. أظهرت أكباد الدجاج والبط المختبرين تبايناً تفاضلياً معنوياً، لكن التباين العضلي كان ضئيلاً إحصائياً. يعد وجود التلوث في كل عينة خطراً على الصحة، ويبدو أن هناك حاجة إلى مزيد من الأبحاث.