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Incidence of Aflatoxins in Poultry Meat and Giblets

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Abstract
Aflatoxins are one of the most dangerous, toxic, teratogenic, and carcinogenic residues in various foods including poultry. This study was conducted to assess the prevalence of aflatoxins in poultry meat, skin, and liver. A total of 80 random samples of different poultry carcasses were collected from 30 carcasses each of 10 fresh broiler carcasses, 10 fresh native poultry carcasses and 10 frozen broiler carcasses represented by 10 muscle samples of each fresh broiler, fresh native and frozen broiler poultry, 10 skin samples of each fresh broiler, fresh native and frozen broiler poultry, and 10 liver samples of only fresh broiler and fresh native poultry. All samples were collected from random retail shops at Beni Suef Governorate to assess the prevalence of Aflatoxins B1, B2 and G2 as well as to compare the levels of contamination among different types of products and poultry breeds. The obtained results clarified that the examined fresh broiler samples showed higher rates of contamination than those of fresh native and frozen broilers carcasses. Whereas liver samples displayed higher levels of aflatoxins when compared with muscles and skin samples. The results were discussed from a hygienic point of view and compared with the international standards to assess their reliability for consumption. In conclusion, poultry carcasses sold in retail markets at Beni Suef governorate contain considerable levels of aflatoxins which could have some public health risks to consumers which may need further investigation to determine the safety of these products.

Keywords
Aflatoxins, Broilers, Giblets, Mycotoxins, Poultry Meat

1. Introduction
Even though chicken giblets and meat make up a sizable amount of the average person's diet, they also pose a serious risk to consumers' health due to mycotoxin exposure and other chemical and biological hazards. Since they are so common in the natural world, Meat and meat products can become contaminated by fungi in several ways. **Adeyeye and Fatih (2016)** state that the main sources of meat contamination by fungi are believed to be the butcher shops' and poultry slaughterhouses' environments, which include the walls, floors, utensils, hides, and the intestinal contents of food animals, in addition to tables, knives, and refrigerators.

Mycotoxins are toxic compounds produced by fungi. **Elzupir and Abdulkhair (2020)** describe them as a diverse group of potent pharmacological and toxic effects on humans and animals, exhibiting heterogeneity. Although more than 300 secondary metabolites have been found, only about 30 pose a threat to the health of people and animals **(Bennett and Klich, 2003).**

Setting reasonable regulatory limits for mycotoxins is one of the most effective ways to protect the public's health because these toxins can contaminate food and feedstuffs and pose a risk to both humans and animals. As a result, **FAO (1997)** established guidelines regarding the permitted levels of mycotoxins present in food and feed products as well as in raw materials.

In terms of structure, mycotoxins are a broad group of complex metabolites produced by fungi, which can be toxic to both humans and animals. Consumers may be exposed to mycotoxins in two ways: 1- The direct method, which involves eating meat that has been spoiled by fungi as well as other plant commodities like cereals, nuts, or fruits. 2- It is known that indirect exposure happens when harmful mycotoxin residues remain in the meat, other tissues, and milk of animals and birds that were fed mycotoxin-contaminated feed **(Fink-Gremmels, 1992).**

Regulatory authorities and agencies and numerous researchers have become aware of the dangerous consequences of mycotoxins in the past few decades. There are three primary causes for this: the first is the impact of mycotoxins on human health. Second, tainted feeds and decreased livestock productivity result in enormous financial losses. Thirdly, because of how mycotoxin contamination affects global commodity trade. Therefore, it is critical for feed manufacturers, livestock producers, and public health to control mold growth and mycotoxin production **(Akande et al., 2006).**

Aspergillus flavus and most strains of Aspergillus parasiticus are the main producers of aflatoxins, which are among the most harmful mycotoxins that contaminate food and feed. Although the liver is the main organ involved in the metabolism of aflatoxin, aflatoxin residues have been discovered in edible tissues such as the muscles, skin, and liver of animals and birds **(Gourama and Bullerman, 1995).** As a result, the active metabolites of AFB1, AFB2, AFG1, and AFG2 would bind to the protein and nucleic acids near the cellular activation sites, causing them to remain in the liver cells **(Carvajal-Moreno, M. 2015).**

There are up to twenty analogs of aflatoxins, but only the following are toxicologically significant: B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), MI (AFM1), and M2 (AFM2).

AFG2 < AFB2 < AFG1 < AFM1 < AFB1, in ascending order of toxicity. The most toxic fraction among all analogs is AFB1, which is found in food and feed products as well as cultures. It is linked to hepatocellular cancer **(Chepkosgei K.R. et al., 2023).**

Therefore, this study was carried out to determine the prevalence of aflatoxins B1, B2 and G1 in poultry edible tissues, as well as to compare the levels of contamination among different types of products and poultry breeds.

2. Material and Methods

2.1. Samples Collection

In all, 80 samples of chicken-meat, skin and liver from sporadic retail establishments in the Beni Suef Governorate, Egypt were examined for the incidence of aflatoxins. 10 samples of each muscle, skin, and liver from each fresh broiler and fresh native carcasses and 10 samples of only muscle and skin from frozen broilers carcasses. Without any delay, the samples were moved straight into the aseptic laboratory. Following homogenization, all samples were frozen in the dark at -20ºC until analysis time.

2.2. Aflatoxins Extraction

According to **Abd El Monem et al. (2015)** the extraction of total aflatoxin residues from tissues was done. While according to **Kalantari et al. (1999)** Solid Phase Extraction (SPE) and derivatization steps were carried out, and then using the High-Performance Liquid Chromatography (HPLC) by injection of 20µL of extract was injected into the device. **(Anklam et al., 2002).**

2.3. Aflatoxins Determination

Equipment and Apparatus: HPLC (Agilent Series 1200) using a fluorescence detector. The chromatographic separation was performed with a reversed-phase column (Extend-C18, Zorbax column, 4.6mm i.d., 250mm, 5µm, Agilent Co.). SPE columns: -Bond Elute C18.HLB Oasis cartridges (6ml), Electro non-digital balance, Mincer, Shaker, nitrogen evaporator, vacuum manifold, and acrodiscs (0.45µm).

To achieve the optimum resolution of the aflatoxins injection, specific liquid chromatographic conditions must applied. Volume 20µl, flow rate of 1.0mL/min, and fluorescence detection was carried out at excitation at 360nm and emission at 440nm. Also the column temperature was at 30ºC.

According to **Abd El Monem et al. (2015)** liquid Chromatographic mobile phase: Isocratic mode using 60:20:20 water/methanol/ acetonitrile mixture as the mobile phase.

2.4. Statistical Analysis

The obtained results were statistically examined by the SPSS 20 **(SPSS Inc, Chicago, IL, USA). (One-way ANOVA)** as per **Sabine and Brian (2004)**.

3. Results

Table 1. Prevalence of Aflatoxins in fresh broilers samples (n=10).

Type of Aflatoxin	Type of sample	NO. of +ve samples	% of +ve samples	Min. (µg/kg)	Max. (µg/kg)	Mean± SEM
B1	SKIN	8	80%	0	1.37	0.291^{b}±0.141
	MUSCLE	3	30%	0	0.197	0.061^{b}±0.030
	LIVER	9	90%	0	18.5	3.772^{a}±2.247
B2	SKIN	0	0%	0	0	0.000^{b}±0.000
	MUSCLE	0	0%	0	0	0.000^{b}±0.000
	LIVER	2	20%	0	2.1	0.291^{a}±0.260
G1	SKIN	0	0%	0	0	0.000^{b}±0.000
	MUSCLE	0	0%	0	0	0.000^{b}±0.000
	LIVER	6	60%	0	3.3	0.685^{a}±0.398

According to FDA, food for human consumption is contaminated if it contains more than 20 micrograms per kilogram (µg/kg) or parts per billion (ppb) of aflatoxins.
Different small letters (a, b, c,…) superscripts within mean column indicate significant differences between means of each aflatoxin type in skin, muscle and liver samples at p<0.05.
SEM= standard error of mean

Table 2. Prevalence of Aflatoxins in fresh native samples (n=10).

Type of Aflatoxin	Type of sample	NO of +ve samples	% of +ve samples	Min. (µg/kg)	Max. (µg/kg)	Mean± SEM
B1	SKIN	3	30%	0	0.57	0.085^{b}±0.057
	MUSCLE	3	30%	0	0.27	0.050^{b}±0.032
	LIVER	6	60%	0	2.525	0.632^{a}±0.269
B2	SKIN	0	0%	0	0	0.000^{a}±0.000
	MUSCLE	0	0%	0	0	0.000^{a}±0.000
	LIVER	0	0%	0	0	0.000^{a}±0.000
G1	SKIN	0	0%	0	0	0.000^{b}±0.000
	MUSCLE	0	0%	0	0	0.000^{b}±0.000
	LIVER	1	10%	0	0.32	0.032^{a}±0.032

According to FDA, food for human consumption is contaminated if it contains more than 20 micrograms per kilogram (µg/kg) or parts per billion (ppb) of aflatoxins.
Different small letters (a, b, c,…) superscripts within mean column indicate significant differences between means of each aflatoxin type in skin, muscle and liver samples at p<0.05.
SEM= standard error of mean

Table 3. Prevalence of Aflatoxins in frozen broiler chicken samples (n=10).

Type of Aflatoxin	Type of sample	NO. of +ve samples	% of +ve samples	Min. (µg/kg)	Max. (µg/kg)	Mean± SEM
B1	SKIN	8	80%	0	1.37	0.000^{a}±0.000
	MUSCLE	3	30%	0	0.197	0.000^{a}±0.000
B2	SKIN	0	0%	0	0	0.000^{a}±0.000
	MUSCLE	0	0%	0	0	0.000^{a}±0.000
G1	SKIN	0	0%	0	0	0.000^{a}±0.000
	MUSCLE	0	0%	0	0	0.116^{b}±0.054

According to FDA, food for human consumption is contaminated if it contains more than 20 micrograms per kilogram (µg/kg) or parts per billion (ppb) of aflatoxins.
Different small letters (a, b, c,…) superscripts within mean column indicate significant differences between means of each aflatoxin type in skin, muscle and liver samples at p<0.05.
SEM= standard error of mean

Table 4. Comparative between Mean ± SEM of Aflatoxins in different types of samples (n=80).

Type of Aflatoxin	Type of sample	Fresh broiler Mean± SEM	Fresh native Mean± SEM	Frozen broiler Mean± SEM
B1	SKIN	0.291^{a}±0.141	0.085^{a}±0.057	0.000^{b}±0.000
	MUSCLE	0.061^{a}±0.030	0.050^{a}±0.032	0.000^{b}±0.000
	LIVER	3.772^{a}±2.247	0.632^{b}±0.269	-
B2	SKIN	0.000^{a}±0.000	0.000^{a}±0.000	0.000^{a}±0.000
	MUSCLE	0.000^{a}±0.000	0.000^{a}±0.000	0.000^{a}±0.000
	LIVER	0.291^{a}±0.260	0.000^{a}±0.000	-
G1	SKIN	0.000^{a}±0.000	0.000^{a}±0.000	0.000^{a}±0.000
	MUSCLE	0.000^{b}±0.000	0.000^{b}±0.000	0.116^{a}±0.054
	LIVER	0.685^{a}±0.398	0.032^{b}±0.032	-

According to FDA, food for human consumption is contaminated if it contains more than 20 micrograms per kilogram (µg/kg) or parts per billion (ppb) of aflatoxins.
Different small letters (a, b, c,...) superscripts within the same row indicate significant differences between means of each aflatoxin type in skin, muscle and liver samples at p<0.05.
SEM= standard error of mean

Furthermore, we investigate the results shown in **(Table 1)** investigate the prevalence of Aflatoxin in fresh broilers breeds chicken samples. The reported results confirmed that B1 was the most prevalent type that percent of +ve samples reach 90% in liver samples, 80% in skin samples and 30% in muscle samples while it was 20% in B2 in liver samples and 0% in each skin and muscle samples, however it was 60% G1 in liver samples and 0% in each skin and muscle samples. The means of B1, B2 and G1, in examined samples from fresh broiler chicken carcasses residues in examined skin samples were 0.291±0.141, 0.000±0.000, and 0.000±0.000 (µg/kg) respectively, while in examined muscle samples from same carcasses were 0.061±0.030, 0.000±0.000, and 0.000±0.000 (µg/kg) respectively, and in liver samples were 3.772±2.247, 0.291 ±0.260, and 0.685±0.398 respectively.

The prevalence of Aflatoxin in fresh native breeds chicken samples **(Table 2).** The reported results showed also that B1 was the most prevalent type of aflatoxin that percent of +ve samples reach 60% in liver samples and 30% in each skin and muscle samples while it was 0% in B2 in all same samples, however it was 10% G1 in liver samples and 0% in each skin and muscle samples. The means of B1, B2 and G1, residues in examined samples from native chicken carcasses residues in examined skin samples were 0.085±0.057, 0.000±0.000, and 0.000±0.000 (µg/kg) respectively, while in examined muscle samples from same carcasses were 0.050±0.032, 0.000±0.000, and 0.000±0.000 (µg/kg) respectively, and in liver samples were 0.632±0.269, 0.000± 0.000, and 0.032±0.032 respectively.

The prevalence of Aflatoxin in frozen broilers chicken samples was investigated in **(Table, 3).** It is obvious from the obtained results that B1 was the most prevalent type of aflatoxin that percent of +ve samples reach 80% in skin samples and 30% in muscle samples while it was 0% in B2 and G2 in the same samples. The B1, B2, G1 aflatoxins residues in examined skin samples in frozen broilers carcasses were not detected, while the same residues in examined muscle samples from same carcasses were 0.000±0.000, 0.000±0.000, and 0.116±0.054 (µg/kg) respectively.

4. Discussion

Several Aspergillus species, primarily A. flavus, produce aflatoxins as secondary metabolites, which are extremely poisonous, mutagenic, teratogenic, and carcinogenic substances **(Zaki et al., 2017).**

As shown in **(Table, 4)** the concentration of AFB1 was the highest among other aflatoxins however, If the total aflatoxins in human food are more than 20 micrograms per kilogram (µg/kg) or parts per billion (ppb), the FDA may consider the food to be contaminated.

This matches with **Zaki et al. (2017)** in which the means of AFB1 reached 5.33±1.8 in turkey liver and 2.31±0.88 in muscles. Also, according to **Ndagijimana et al. (2002)** it is the most significant aflatoxin now in existence among a variety of other mycotoxin types, which have been shown to contaminate a significant portion of the world's food supply and cause a worldwide issue with food insecurity.

The results revealed higher residual levels of aflatoxin residues in examined liver samples than that of examined skin and muscle samples. Moreover, 17 samples of examined fresh broiler liver samples were positive compared with 8 and 3 samples for examined skin and muscles respectively. This may be attributed to liver tissues being considered the main reservoir for aflatoxins. The recorded results in the present study agreed with those obtained by **Herzallah (2013)** and **Darwish et al. (2016)** who found that frozen livers had the highest aflatoxin residues followed by frozen gizzards, breast, and thigh-cuts. Also, **Zaki et al. (2017)** as they found that the examined liver samples were contaminated with a higher concentration of AFB1 and total aflatoxins than in kidneys, gizzard, thigh and breast samples. Moreover, **Morshdy (2015)** found that AFB1 constituted the highest level among aflatoxins detected, followed by AFG1 in examined chicken liver and fillet samples. This agreed with the results in the current study. AFB1 is extremely considered carcinogenic type of aflatoxins that is responsible for carcinogenicity of human beings as mentioned by World Health Organization **(Anklam et al., 2002).** Some Aspergillus species have received a great attention as they can produce aflatoxins which have potential hazards to consumers through their mutagenic, carcinogenic, hepatotoxic, immunosuppressive **(Shephard, 2008)** and teratogenic effects **(Probst et al., 2007)** and resulted in acute hepatitis B and C **(Ramesh and Siruguri, 2003),** liver cirrhosis, acute liver damage, and hepatic cancer in human being **(Probst et al., 2007).** The obtained results were higher than that recorded by **Hussain et al. (2016).** In the contrary, **Abo El-Yazeed et al. (2015)** detected higher aflatoxins residues in breast muscles than that recorded in examined liver samples. Several environmental factors affect the biosynthesis of aflatoxins by molds including temperature, humidity, light and pH as mentioned by **Hesseltine (1983).** In this respect, bad sanitary measures applied in abattoirs including contaminated water, bad ventilation, contaminated tools, mishandling of carcasses, and improper evisceration lead to increase the probability of molds contamination and subsequently aflatoxins production. Except for milk, which has an action level of 0.5ppb of aflatoxin M1, the Food and Drug Administration **(FDA, 2011)** issued regulatory working guidelines on the allowable levels of aflatoxins in human meals. These standards are set at 20ppb for total aflatoxins. However, it should be noted that improper food production and handling practices may accelerate the production of aflatoxins. At the same time, most mean values of detected aflatoxins in the examined samples were lower than the maximum permissible limit recommended by the European Community (EC) No 1881/2006 in food for human consumption of 10µg/kg for total aflatoxins B1, B2, G1, and G2. General review of the three tables will show that results of fresh broilers samples showed higher residual levels than fresh native and frozen broilers samples.

5. Conclusion

The availability of cheap rates of chicken giblets and meat has led to an increase in their consumption. But the high amounts of Afs seen in chicken giblets and meat, particularly in the liver of fresh broilers, are concerning for human health. As a result, quick action is needed to keep an eye on and manage these contaminants in chicken products. It is important to adhere to the stringent allowable limits in order to prevent

fungal contamination. Strict hygienic practices, such as applying the HACCP system and GMP and handling poultry meat and feed through all stages, are the most effective way to prevent aflatoxigenic mold contamination of poultry products. It is imperative that workers and handlers participate in educational programs and training courses. Additionally, in order to obtain foods free of mycotoxins, particularly aflatoxins, markets that slaughter, prepare, and sell poultry carcasses as retailed parts should adhere to Good Manufacturing Practices (GMP), Good Sanitary Practices (GSP), and Good Hygiene Measures (GHM). People who handle poultry products must be well-trained in the preventive control programs and aware of potential hazards in order to guarantee the product's safety.

6. Authors Contributions

All authors participated equally to the design of the research, methodology, and writing of the manuscript.

7. Conflict of Interest

The authors declare no conflict of interest.

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