

Gamma radiation reduces the levels of aflatoxins B₁ in poultry meat, skin, and liver

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ARTICLE INFO

Received: 07 January 2024

Accepted: 19 January 2024

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Keywords:

Poultry
Broilers
Gamma Radiation
Mycotoxins
Aflatoxins

ABSTRACT

Aflatoxins are one of the most dangerous toxic residues in various foods including poultry. This study was conducted to assess the reducing effect of gamma radiation on the levels of aflatoxin B₁ in poultry meat, skin, and liver. To this end, a total of 80 poultry samples including meat, skin, and liver were surveyed for the incidence of aflatoxins, where only positive samples (27 samples of muscle, skin, and liver; 9 samples each) were selected for testing the effect of treatment by gamma radiation. The levels of aflatoxins were estimated in the examined samples using High Performance Liquid Chromatography (HPLC) whereas positive samples were exposed to 0 kGy, 5 kGy, or 10 kGy, and the differences in aflatoxin contents before and after exposure were calculated. The obtained results clarified that radiation achieved reduction rates in aflatoxin B₁ level in muscle samples with a mean value of 99.259 ± 0.741 , and $100.00 \pm 0.00\%$ when treated with 5 kGy and 10 kGy, respectively. Whereas in skin samples, 98.676 ± 1.324 and $100.00 \pm 0.00\%$ when treated 5 kGy and 10 kGy, respectively. In liver samples, the reduction rates accounted for 84.312 ± 7.406 and 88.249 ± 10.882 were obtained when treated with 5 kGy and 10 kGy, respectively. In conclusion, the exposure of poultry meat, skin, and liver to gamma radiation (5 kGy or 10 kGy) has a significant reducing effect ($p < 0.05$) in aflatoxins B₁. The results were discussed from the hygienic point of view and compared with the national and international standards to assess their reliability for consumption.

Introduction

Although poultry meat and giblets represent an important portion of the daily human diet, they constitute significant sources of biological hazards and chemical hazards to consumers including mycotoxins. Fungi are widely distributed in nature and may contaminate meat and meat products in several ways. The poultry slaughterhouses environment and butcher shops including walls, floors, utensils, hides and the intestinal contents of food animals, as well as tables, knives, and refrigerators, are considered the main sources of fungal contamination of meat (Adeyeye and Fatih, 2016).

Mycotoxins are toxic substances developed by fungi. They constitute a heterogeneous group of secondary metabolites with diverse potent pharmacological and toxic effects on humans and animals. More than 300 secondary metabolites have been identified but around 30 are of real concern to human and animal health (Bennett and Klich, 2003).

Mycotoxins can contaminate food and feedstuffs and these contaminated materials may be dangerous to animals and humans; therefore, one of the most effective measures to protect public health is to establish reasonable regulatory limits for these toxins. Consequently, guidelines regarding the allowed levels of mycotoxins present in food and feed products and in raw materials have been established by FAO (1997).

Mycotoxins structurally comprise a diverse family of fungal-elaborated metabolites, which can induce toxicity in humans and animals. Myco-

toxins may reach consumers in two different ways: (1) The direct route via ingestion of cereals, nuts or fruits, and other plant commodities as well as meat that are spoiled by fungi. (2) An indirect exposure is known to occur when toxic residues of mycotoxins persist in meat and other tissues as well as milk from animals and birds, which were exposed to feedstuff contaminated with mycotoxins (Fink-Gremmels, 1992).

The hazardous effects of mycotoxins have attracted the attention of many researchers and regulatory authorities over the last few decades. This is due to three main reasons: firstly, the effect of mycotoxins on human health. Secondly, due to the huge economic losses associated with contaminated feeds and the loss of livestock productivity. And thirdly due to the impact of mycotoxin contamination on international trade in commodities. So, controlling mold growth and mycotoxin production is very important to public health, feed manufacturers, and livestock producers (Akande *et al.*, 2006).

Out of mycotoxins, aflatoxins are one of the most dangerous toxins contaminating foods and feed, they are produced primarily by some strains of *Aspergillus flavus* and most strains of *Aspergillus parasiticus*. Aflatoxins were found as residues in edible tissues, including the liver, skin, and muscles of animals and birds, whereas the liver is the principal organ of aflatoxin metabolism (Gourama and Bullerman, 1995). Accordingly, the active metabolites of AFB₁, AFB₂, AFG₁, and AFG₂ would bind to protein and nucleic acids in the vicinity of the cellular activation sites and be retained in the liver cells (Carvajal-Moreno, 2015).

Various studies further suggest that the total elimination of moulds and their toxins is practically impossible, so there is a great need for the use of agents that are able to bind the toxins selectively in the gut, thus limiting their bioavailability in the consumers. In addition, the possible presence of toxic residues in poultry products (egg, meat), which enter the food chain may pose potential risks by their detrimental effects on human health (Patil *et al.*, 2014).

AFs are highly resistant to heat treatment since their decomposing temperature is higher than 235°C, therefore, simple drying cannot decrease their concentrations in stored grains significantly. However, long-time high-temperature treatments seem to have a beneficial effect on decontamination and due to the adverse effects of heat treatments on nutritional properties, the food industry is increasingly interested in non-thermal technologies (Sipos *et al.*, 2021). Also, Ammonization despite its efficacy can lead to a food quality decrease and deterioration due to excessive ammonia levels in the food (Čolović *et al.*, 2019).

On the other hand, some studies show efficient reduction (Al-Ruwaili *et al.*, 2018). The authors stated that yogurt whey (which contained live lactic acid bacteria) in drinking water was able to significantly reduce AFB1 to low levels in meat and organ tissues of broiler chickens. Reduction of aflatoxin AFB1 accumulation in broiler chicken organs or tissues most likely occurred because of direct biodegradation of AFB1 by LAB in the gastrointestinal tract.

To study the incidence of aflatoxins in edible poultry meat and tissues, randomly collected poultry samples including meat, skin, and liver were examined for the incidence of aflatoxins, and then positive samples were selected for this study. The current study aimed to investigate the reducing effect of gamma radiation treatment on the levels of aflatoxin in positive samples.

Materials and methods

Collection of samples

A total of 80 poultry samples including meat, skin, and liver were surveyed for the incidence of aflatoxins from random retail shops in Beni-Suef Governorate, Egypt. The samples were transferred directly to the laboratory under aseptic conditions without delay. Afterward, all samples were homogenized and frozen at -20°C in the dark till the time of analysis.

Extraction of aflatoxins

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

Determination of aflatoxins

Apparatus and equipment: HPLC (Agilent Series 1200) using a fluorescence detector. The chromatographic separation was performed with a reversed-phase column (Extend-C18, Zorbax column, 4.6 mm i.d., 250

mm, 5 µm, Agilent Co.). SPE columns: -Bond Elute C18.HLB Oasis cartridges (6 ml), Electro non-digital balance, Mincer, Shaker, nitrogen evaporator, vacuum manifold, and acrodiscs (0.45µm).

Liquid chromatographic conditions to achieve the optimum resolution of the aflatoxins injection volume 20 µl, flow rate of 1.0 mL/min. The column temperature was at 30°C, and fluorescence detection was carried out at excitation at 360 nm and emission at 440 nm. Liquid Chromatographic mobile phase: Isocratic mode using 60:20:20 water/methanol/acetonitrile mixture as the mobile phase according to Abd El Monem, *et al.* (2015).

Radiation treatment

Twenty-seven samples of poultry samples containing aflatoxins based on the preliminary study, consisting of 9 samples each of muscle, skin, and liver were transferred to the gamma radiation laboratory in Atomic Energy Authority, Cairo under aseptic conditions. Samples were exposed to gamma radiation at doses of 0 kGy, 5 kGy or 10 kGy using ⁶⁰Co γ rays (Gamma Cell mold 220 apparatus, NCRRT, Nasr City, Cairo, Egypt). According to the FAO/IAEA/WHO Expert Committee on Food Irradiation, "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard, hence, toxicological testing of food so treated no longer required." Joint FAO/IAEA/WHO Expert Committee (1981).

Retesting

A total of 27 poultry samples both those exposed to gamma radiation (5 kGy and 10 kGy), as well as untreated one (0 kGy) were retested according to the aforementioned methods in 2.2. and 2.3. to estimate the difference in aflatoxin contents in comparison to the levels before treatment.

Statistical Analysis

The obtained results were statistically analyzed using Statistical Package for Social Sciences (SPSS). One Way ANOVA was conducted according to Sabine and Brian (2004).

Results

The effect of γ radiation on the levels of aflatoxin B1 levels in muscle samples of broilers chicken was investigated (Table 1). It is obvious from the obtained results that there was no obvious reduction in the levels of aflatoxin B1 in control (non-irradiated) muscle samples between the two measurements. On the other hand, gamma irradiation with 5 kGy induced significant reductions ($p < 0.05$) ranging from 97% to 100% with a mean value of 99.259% in the levels of aflatoxin B1 in treated muscle samples. Additionally, the reduction rate in treated broiler muscle samples with 10 kGy was 100%. Significant differences between means of control and irradiated samples (5 kGy and 10 kGy) after irradiation were noticeable ($p < 0.05$). Furthermore, significant differences between means of the same group before and after irradiation ($p < 0.05$) were detected.

Furthermore, we estimated the effect of gamma radiation on the lev-

Table 1. Effect of γ radiation on aflatoxin B1 levels in broiler chicken muscle samples.

Treatments	Aflatoxin B1 level before radiation treatment (µg/kg)	Aflatoxin B1 level after radiation treatment (µg/kg)	Reduction rate (%)
Control (untreated)	0.182±0.008	0.176±0.014 ^a	3.92±3.92
Irradiated with 5 kGy	0.182±0.008	0.001±0.001 ^{b*}	99.259±0.741
Irradiated with 10 kGy	0.182±0.008	0.00±0.00 ^{b*}	100.00±0.00

Data are presented as Mean± SEM.

Different small letter superscripts (a, b, c, ...) indicate significant differences between means of control and irradiated samples after irradiation at $p < 0.05$. The asterisk symbol (*) indicates significant differences between aflatoxin B1 levels before and after irradiation at $p < 0.05$.

Table 2. Effect of γ radiation on aflatoxin B1 levels in broiler chicken skin samples.

Radiation dose	Aflatoxin B1 level before radiation treatment ($\mu\text{g}/\text{kg}$)	Aflatoxin B1 level after radiation treatment ($\mu\text{g}/\text{kg}$)	Reduction rate%
Control (untreated)	0.085 \pm 0.028	0.047 \pm 0.017 ^a	27.45 \pm 27.45
Irradiated with 5 kGy	0.169 \pm 0.091	0.005 \pm 0.005 ^{b*}	98.676 \pm 1.324
Irradiated with 10 kGy	0.942 \pm 0.428	0.000 \pm 0.000 ^{b*}	100.00 \pm 0.00

Data are presented as Mean \pm SEM.

Different small letter superscripts (a, b, c, ...) indicate significant differences between means of control and irradiated samples after irradiation at $p < 0.05$. The asterisk symbol (*) indicates significant differences between aflatoxin B1 levels before and after irradiation at $p < 0.05$.

Table 3. Effect of γ radiation on aflatoxin B1 levels in broiler chicken liver samples

Radiation dose	Aflatoxin B1 level before radiation treatment ($\mu\text{g}/\text{kg}$)	Aflatoxin B1 level after radiation treatment ($\mu\text{g}/\text{kg}$)	Reduction rate%
Control (untreated)	0.463 \pm 0.169 ^a	0.439 \pm 0.177 ^{a*}	7.599 \pm 3.802
Irradiated with 5 kGy	2.840 \pm 2.030 ^b	0.460 \pm 0.336 ^{b*}	84.312 \pm 7.41
Irradiated with 10 kGy	7.297 \pm 5.605 ^c	2.076 \pm 2.062 ^{b*}	88.249 \pm 10.88

Data are presented as Mean \pm SEM.

Different small letter superscripts (a, b, c, ...) indicate significant differences between means of control and irradiated samples after irradiation at $p < 0.05$. The asterisk symbol (*) indicates significant differences between aflatoxin B1 levels before and after irradiation at $p < 0.05$.

els of aflatoxins B1 in skin samples of broiler chicken (Table 2). The reported results showed that the reduction rate of untreated broilers breeds skin samples ranged from 0% to 82% with a mean value of 27.451. While reduction rate of treated broilers breeds skin samples by 5 kGy ranged from 96% to 100% with a mean value of 98.676 \pm 1.32 and reduction rate of treated broilers breeds muscle samples by 10 kGy was 100% with a mean value of 100.00 \pm 0.00.

The results shown in Table 3 confirmed that the mean values of aflatoxin B1 in the untreated liver samples were 0.463 and 0.439 $\mu\text{g}/\text{kg}$ without significant reductions in the levels referring to the time before and after irradiation of treated samples. On the other hand, treated broiler liver samples by 5 kGy displayed a reduction rate ranged from 72% to 84% with a mean value of 84.31, while treatment with 10 kGy induced a reduction rate in aflatoxins B1 in treated broiler liver samples ranged from 66% to 99% with a mean value of 88.249 \pm 10.882. The mean values of aflatoxins B1 in skin samples treated with 5 kGy were 2.840 and 0.460 before and after irradiation, respectively. While the mean values in liver samples treated with 10 kGy were 7.297 and 2.076 before and after irradiation, respectively. These results highlighted the significant reducing effects of gamma irradiation on the levels of aflatoxins B1 in liver samples of broilers ($p < 0.05$).

Discussion

In the present study, the highest levels of aflatoxins B1 were recorded in liver samples, followed by skin samples, while muscle samples had the lowest levels of these residues. FDA may consider human food containing total aflatoxins greater than 20 micrograms per kilogram ($\mu\text{g}/\text{kg}$) or parts per billion (ppb) to be adulterated (FDA, 2011). In this regard, none of the examined samples had this high level of aflatoxins.

Modern food preservation techniques include food irradiation, a physical procedure that uses ionizing radiation to stop the development of undesirable living organisms or to decrease their population (Ferreira-Castro *et al.*, 2007). Food irradiation is a promising technology that has the potential to improve both the quality and the safety of meat (Indiarto *et al.*, 2023).

International organizations like the World Health Organization (WHO), the Food and Agriculture Organization (FAO) of the United Nations, the International Atomic Energy Agency (IAEA), and Codex Alimentarius have all approved the safety of irradiation technology. Food irradiation has the potential to increase food's shelf life while preserving its organoleptic and nutritional qualities (Iqbal *et al.*, 2013).

The obtained results showed a positive correlation between the reduction rate of aflatoxins B1 in all examined samples and the dose of gamma irradiation applied to the samples. As the highest aflatoxin reduction rate was attained at 10 kGy; it reached 100% in muscle and skin with a mean value of 100% for both, followed by 88.24% in liver samples. These findings are in harmony with Abd El-Tawaab *et al.* (2019), whereby the most reduction percentage of mycotoxins were achieved at 10 kGy, which it reached 19.6% for total mycotoxins, 27% for AF B1, 40.43% for

AF B2, 59.42% for AF G1, 92.15% for AF G2, and 73.44% for ochratoxins A. Also, they are in consistent with Awad *et al.* (2019), who found that at 10 kGy the maximum reduction percentage of mycotoxins was attained. It reached 27.22% for AFB1 and agrees also with El-Yazeed *et al.* (2015) who found that in the dose of 10 kGy the degradation of AFB1 reached the highest values at 87.8, 81.1, 84.6, 68.8, and 58.6% for rice samples, corn, peanuts, unpeeled pistachios, and peeled pistachios, respectively. Furthermore, according to Ghanem *et al.* (2008), the greatest percentages of AFB1 degradation at 10 kGy dose were 58.6, 68.8, 84.6, 81.1, and 87.8% for the samples they tested. On the other hand, Vita *et al.* (2014) claimed that the maximum reduction rate was achieved at 15 kGy, which was 19.25% for AFB1, while the maximum reduction in AFB1 according to Elbarbary *et al.* (2023) reached 79% by 15 kGy.

In the current study, similar results were found in samples treated by 5 kG, as the highest aflatoxin reduction percentage was attained at muscle and skin with mean values accounting for 99.25 and 98.67%, respectively, followed by liver samples which had a reduction rate accounted for 84.31%. This could be explained by the higher levels of aflatoxins in liver samples than in muscle and skin ones, as the liver is the harbor site for mycotoxins residues (Abd El-Tawaab *et al.*, 2019).

These findings suggest that aflatoxins in highly contaminated food may be degraded by gamma irradiation to concentrations within safe levels and could become fit for human consumption. Similarly, Herzallah *et al.* (2008) reported that γ -irradiation significantly ($P < 0.05$) affected the concentrations of AFB1 and total aflatoxin in poultry feed samples and the degradation rate increased with increased irradiation dose. Also, according to Jalili *et al.* (2010), the average recovery values of aflatoxins ranged from 72% for aflatoxins AFG2 to 101% for ochratoxins A and the obtained recoveries for all mycotoxins were in line with the permissible levels described by the European Commission (EC) in 2006.

Furthermore, Refai *et al.* (2003) reported that in a dose level of 3 kGy, only one sample each of pepper, fenugreek, and spice paste were contaminated with aflatoxins, and in 5 kGy, all basterma samples and their components were free from aflatoxins.

On the contrary, according to results obtained by Di Stefano *et al.* (2014), gamma rays even at 15 kGy were not effective in the destruction of ochratoxin A and aflatoxins in the tested feed.

Conclusion

Food irradiation had a significant reducing effect on aflatoxin levels, which requires further investigations to set up suitable approaches and mechanisms to apply in the food industry for food safety purposes. Additionally, it is essential that national and international regulatory authorities regulate irradiation use in food processing, and they state labelling requirements for irradiated food products. It is recommended that policymakers consider incorporating irradiation as a part of a comprehensive food safety strategy. Future research on food irradiation could also find emerging technologies and applications, addressing areas of uncertainty and controversy, as well as improving current knowledge about the effects of irradiation on meat.

Conflict of interest

The authors declare that they have no conflict of interest.

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