Aflatoxin Decontamination of Artificially Contaminated Feeds by Sunlight, γ-Radiation, and Microwave Heating

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Primary Audience: Nutritionists, Ministry of Agriculture, Poultry Producers, Feed Manufacturers

SUMMARY:

The efficiency of decontamination of aflatoxin residues in poultry feeds through exposure to sunlight (solar radiation), γ-radiation (60Co), and microwave heating were investigated in artificially contaminated feed samples. Photodegradation of aflatoxin by sunlight has been found to cause a significant (P<0.05) decrease in both B1 and the total aflatoxins. Moreover, the degrees of aflatoxins were dependent on exposure time. Both aflatoxin B1 and total aflatoxins were decreased when feed samples exposed to sunlight by 42.3, 39.9, 75.5, and 65.9% for 3 and 30 h of direct sunlight of the treatment T1, whereas feed samples subjected to γ-irradiation and microwave heating caused a significant (P<0.05) decrease in aflatoxin B1 contents by 42.7 and 32.3% for γ-irradiation and microwave heating (T3 of 25 kGy and 10 min of microwave heating), respectively. Therefore, the solar radiation was more effective in aflatoxin B1 reduction when compared with γ-irradiation and microwave heating.

Key words: aflatoxin, microwave, sunlight, γ-radiation, feed
DESCRIPTION OF PROBLEM:

Mycotoxins are fungal metabolites that diffuse into feed or food. Aflatoxins B1 (AFB1) and total aflatoxins (B1, B2, G1, and G2) are considered as highly toxic and carcinogenic compounds for human and animals [1] produced mainly by molds, Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius [2]. These fungi can grow on a wide range of agricultural food and feed products. They are of worldwide importance, causing problems in public health, agriculture, and high economic losses [3, 4]. The lack of preharvest control of mycotoxins has made them a postharvest threat in feed products. Mycotoxin contamination is sometimes referred to as ineluctable; therefore, the preventive measures must be placed as a long-term goal to prevent health hazards, economical implication with potential loss, and increase interest for lowcost feed materials that may cause a decrease in the price of animal products. Various mycotoxin decontamination methods have been reported, including physical, chemical [5–7], or biological [2, 8] methods. Physical process involves separation of the contaminated parts, removal of mycotoxins, and inactivation of mycotoxins by physical means, such as heat, cooking, roasting, and radiation [9, 10]. The process of physical, chemical, and microbiological methods must ensure that the degradation process retains the nutritive value, will not result in the introduction of new toxic or carcinogenic-mutagenic substances, and the process must also destroys Aspergillus spores and mycelia that could, under favorable conditions, proliferate and produce new toxins [11–13]. Chemical degradation of toxins with chlorine, hydrogen peroxide [13], ozone and ammonia, alkali, and acids have been investigated for their destruction power for AFB1. The chlorine, hydrogen peroxide, and ozone are considered as less satisfactory, and bisulfite has low efficiency with the possibility of toxic epoxide formation [14–16]. Thus, due to the high risks of aflatoxins, the tolerance levels in feed and food were determined at very low concentrations. The action concentrations set by the Food and Drug Administration is 20 ppb for aflatoxin for dairy cow
and 100 ppb for other feed [17]. The tolerance concentrations for aflatoxins in foodstuffs are in the range of 5 to 50 ppb [18]. Microwave heating and radiation processing as methods used for preservation of foods and prevention of food from spoilage is finding an application in the food and feed industry as safety controlling tools [19]. Therefore, the present study has been conducted to evaluate the efficacy of sunlight (solar radiation), y-radiation, and microwave heating for the decontamination of AFB1 and total aflatoxins (B1, B2, G1, G2) occurring in chick feed employing enzyme-linked immunosorbent assay (ELISA) in AFB1 and total aflatoxin analysis.

**MATERIALS AND METHODS:**

*Sample Preparation, Sunlight, and Microwave Heating:*

The study involved 4 feed samples analyzed to be free of AFB1 or the total aflatoxin by ELISA. One of the aflatoxin-free samples was considered as the control (C). Three additional treatments, T1, T2, and T3, were artificially contaminated with pure aflatoxins of 965, 421, and 210 μg/kg of total aflatoxins and 894, 395, and 192.1 μg/kg of AFB1 aflatoxin [20]. Aflatoxins were dissolved in 5 mL of methanol of HPLC grade [21] and mixed with 5 kg of feed for 30 min. The mixed feed was left for 24 h then mixed with 20 kg of poultry feed by manual shaking 70 times(10 times/d for 7 d). Control and contaminated feeds were then placed in plastic containers (20-kg capacity) for 1 wk. Feeds were analyzed for aflatoxin after storage. Two feed samples in duplicate of each treatment (C, T1, T2, and T3) were placed in a tray with a thickness of 1 to 2 mm under direct bright sunlight at noon during the summer season of 2006 for 0, 3, 9, and 30 h for an average of 5 h of exposure time per day (solar radiation was not measured). Also, 2 samples of about 100 g each from the same treatments were heated in a microwave oven [22] for 2, 4, 8, and 10 min at 100% power of HF (high frequency) output of 2,450 MHz and 1.45 kW.
\textbf{\textit{\textgamma}-Irradiation:}

Samples of treatment C, T1, T2, and T3 of 200 g were packed into polyethylene bags and irradiated in duplicate with doses of 0, 5, 10, 15, 20, and 25 kGy (52 min/kGy) by using 60Co source [23]. Then the irradiated samples were reevaluated for the retained total and B1 aflatoxin concentration.

\textit{Determination of AFB1 and Total Aflatoxin:}

A 100- to 200-g sample of each feed corresponding to different treatments in duplicate was ground [24] and passed through the mill sieve of 0.25-mm opening, and a control sample was ground between treatments to prevent contamination. The prepared samples were tested using microplate ELISA quantitative test kits [25] and reader [26]. The quantitative analyses of aflatoxin total concentrations and B1 were determined in the prepared samples after storage. The samples (treatments and control) were analyzed using the total aflatoxin test procedure (art. no. R4701), which is described by the RBiopharm test procedure kit manufacturer [25].

\textit{Aflatoxin Working Standards:}

Six working standard dilutions of 0, 0.5, 1.5, 4.5, 13.5, and 40.5 \textmu g/L (ppb) of aflatoxin (provided with the ELISA kit) were used and immunoassayed in triplicate. Also, a zero standard was methanol-diluted in sample buffer (1+9) and assayed in the presence of the enzyme conjugate. Blank wells were the same as zero standards but assayed without the enzyme conjugate.

\textit{Test Protocol:}

A 2-g sample of the ground (0.25-mm sieve), powdered feed samples was mixed in a screwcap glass vial with 10 mL of methanol:distilled water (70:30) mixture and mixed for 30 min at a room temperature of 20 to 25°C using an IKA shaker [24].
The mixture was filtered by using a filter paper (Whatman no. 1). One hundred microliters of the filtrate was diluted with 600 Ml (1 + 6) of the sample dilution buffer (phosphate buffer solution, pH 7.2).

**Table 1.** Effect of sunlight (solar irradiation) on aflatoxin concentrations in contaminated chick feed$^{1,2}$

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Total aflatoxin (μg/kg)</th>
<th>Aflatoxin B1 (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>965$^a$</td>
<td>421$^a$</td>
</tr>
<tr>
<td>3</td>
<td>580$^b$</td>
<td>246$^b$</td>
</tr>
<tr>
<td>9</td>
<td>439$^c$</td>
<td>132$^c$</td>
</tr>
<tr>
<td>30</td>
<td>329$^d$</td>
<td>106.1$^d$</td>
</tr>
</tbody>
</table>

$^a$-$d$Means with the same superscript letter within a column are not significantly different ($P > 0.05$).

$^1$Values are mean of 3 replicates ($n = 3$).

$^2$Concentrations for controls were less than the detection limit of the enzyme-linked immunosorbent assay procedure.

**Total Aflatoxin Analyses:**

Fifty microliters of each buffer-diluted (phosphate buffer solution) standard solution (1 + 9) and prepared diluted samples (1 + 6) were added into separate wells of a microtiter plate in triplicate. Then 50 μL from buffer-diluted enzyme conjugate (1+10) and 50 μL of buffer-diluted antiaflatoxin antibody (1+10) were added and mixed gently for 30 s. The plates were incubated for 30 min at room temperature in the dark. The liquid was poured out of the wells completely and tapped against adsorbent paper 3 times, and then each well was filled with 250 μL of distilled water and the liquid was poured out of the wells. This washing procedure was repeated 2 more times. Subsequently, enzyme substrate (urea peroxide, 50 μL) and chromogen (tetramethyl- benzidine, 50 μL) were added to each well, and the plate was mixed gently for 30 s and incubated for 30 min at room temperature in the dark. To each well, 100 μL of the stop solution (1 N H2SO4) was added, and the absorbance was measured at 450 nm in an ELISA reader [26].
Recovery of Aflatoxins:

The standard curves were linear with a correlation coefficient of 0.998. Means for CV were 1.56% in within-day and 2.06% in between-day analysis for AFB1 and total aflatoxin determinations.

**Table 2.** Effect of solar irradiation on aflatoxin percentage (%) in contaminated chick feed$^{1,2}$

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Total aflatoxin (%)</th>
<th>Aflatoxin B1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>100$^a$</td>
<td>100$^a$</td>
</tr>
<tr>
<td>3</td>
<td>60.1$^b$</td>
<td>58.4$^b$</td>
</tr>
<tr>
<td>9</td>
<td>45.5$^c$</td>
<td>31.3$^c$</td>
</tr>
<tr>
<td>30</td>
<td>34.1$^d$</td>
<td>25.2$^d$</td>
</tr>
</tbody>
</table>

$^a$–$^d$ Means with the same superscript letter within a column are not significantly different ($P > 0.05$).

$^1$ Values are mean of 3 replicates ($n = 3$).

$^2$ Concentrations for controls were less than the detection limit of the enzyme-linked immunosorbent assay procedure.

**Table 3.** Effect of γ-irradiation on aflatoxin in contaminated chick feed$^{1,2}$

<table>
<thead>
<tr>
<th>Radiation dose (KgY)</th>
<th>Total aflatoxin (µg/kg)</th>
<th>Aflatoxin B1 (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>965$^a$</td>
<td>421$^a$</td>
</tr>
<tr>
<td>5</td>
<td>860$^b$</td>
<td>400$^b$</td>
</tr>
<tr>
<td>10</td>
<td>830$^c$</td>
<td>380$^c$</td>
</tr>
<tr>
<td>15</td>
<td>770$^d$</td>
<td>340$^d$</td>
</tr>
<tr>
<td>20</td>
<td>680$^c$</td>
<td>300$^c$</td>
</tr>
<tr>
<td>25</td>
<td>630$^f$</td>
<td>280$^c$</td>
</tr>
</tbody>
</table>

$^{a-f}$ Means with the same superscript letter within a column are not significantly different ($P > 0.05$).

$^1$ Values are mean of 3 replicates ($n = 3$).

$^2$ Concentrations for controls were less than the detection limits of the enzyme-linked immunosorbent assay procedure.
Statistical Analysis:

Data for feed exposed to sunlight, microwave heating, and γ-irradiation were subjected to ANOVA using the GLM procedure in PCSAS version 7.0 [27]. Variable means for measurements showing significant differences in the ANOVA were compared using the LSD procedure. Values were judged to be significantly different by Duncan and LSD if $P < 0.05$

RESULTS:

The photodegradation of aflatoxins was found to increase with increased duration of exposure time (Table 1). Greater degrees of aflatoxin degradation were observed with increasing the length (time) of exposure to solar radiation. The calculated percentages of AFB1 and total aflatoxin degradation when exposed to sunlight are presented in Table 2. More than 60% of the aflatoxin was found to be degraded after 30 h of exposure to sunlight. The extent of degradation was found to be independent of the original aflatoxin concentrations of 421 to 965 μg/kg. The effect of different doses of γ-irradiation on AFB1 and total aflatoxin is shown in Table 3. The data show that the degradation of aflatoxins to some extent was greater by increasing the dose levels from 5 to 25 kGy except for treatment T1 of AFB1. Also, the results show that the treatment of chick feed with the γ-irradiation degraded more than 30% of AFB1 and total aflatoxins in the exposed feed samples. The percentage of aflatoxin destruction when irradiated with γ-irradiation was presented in Table 4. Percentages of aflatoxin (B1 or total) reduction were between 34 to 40% for total aflatoxin and 32 to 42% for AFB1. The effect of microwave heating on aflatoxin concentrations was presented in Table 5. The results show that microwave heating is less effective in decontamination of AFB1 and total aflatoxins. The percentages of reduction were between 22 to 32% (Table 6). The extent of detoxification with microwave heating was found to be less effective as a method of
decontamination under these conditions with a maximum exposure time of 10 min.

**Table 4.** Effect of γ-radiation on aflatoxin percentage (%) in contaminated chick feed

<table>
<thead>
<tr>
<th>Radiation dose (KgY)</th>
<th>Total aflatoxin (µg/kg)</th>
<th>Aflatoxin B1 (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>89.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>86.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>79.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>70.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>71.3&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>65.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>66.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-e</sup>Means with the same superscript letter within a column are not significantly different (P > 0.05).

<sup>1</sup> Values are mean of 3 replicates (n = 3).

<sup>2</sup> Concentrations for controls were less than the detection limits of the enzyme-linked immunosorbent assay procedure.

**Table 5.** Effect of microwave heating on aflatoxin concentrations in contaminated chick feed

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Total aflatoxin (µg/kg)</th>
<th>Aflatoxin B1 (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>965&lt;sup&gt;a&lt;/sup&gt;</td>
<td>421&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>960&lt;sup&gt;a&lt;/sup&gt;</td>
<td>415&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>930&lt;sup&gt;b&lt;/sup&gt;</td>
<td>400&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>800&lt;sup&gt;c&lt;/sup&gt;</td>
<td>380&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>760&lt;sup&gt;d&lt;/sup&gt;</td>
<td>330&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-e</sup>Means with the same superscript letter within a column are not significantly different (P > 0.05).

<sup>1</sup> Values are mean of 3 replicates (n = 3).

<sup>2</sup> Concentrations for controls were less than the detection limits of the enzyme-linked immunosorbent assay procedure.

**DISCUSSION:**

The results presented in this study indicated that the major proportion of aflatoxin reduction resulted from the duration of
the solar irradiation. The degradation of aflatoxin by sunlight has been found to cause a significant decrease in both AFB1 and total aflatoxins. Moreover, the degree of aflatoxin reduction was found to be dependent on the duration of exposure to sunlight. The results were in agreement with the results obtained by Shantha and Sreenivasa Murthy [28], who found a significant \( P < 0.05 \) B1 reduction of 75% of the aflatoxin-contaminated crude groundnut oil after solar irradiation treatment for 10 min. On the other hand, Samarajeewa et al. [29] found that solar irradiation caused the color and fatty acid to be slightly increased. The results also indicated that solar irradiation was not capable of completely destroying the aflatoxins in chick feed. This could be due to the presence of coarser particles that leads to lack of feed particle size homogeneity that would retain a larger proportion of aflatoxins and prevent the complete penetration of solar irradiation to the whole feed samples. This result was in agreement with those found by Basappa and Sreenivasa Murthy [30] and Shantha and Sreenivasa Murthy [31], who found that penetration of radiation into aflatoxin-containing particles is a limiting factor in the process of detoxification by solar irradiation of groundnut oil. \( \gamma \)-Irradiation significantly \( P < 0.05 \) affected the concentrations of AFB1 and total aflatoxin (Table 3). The degradation rate increased with increased irradiation dose. For example, the concentrations of the total aflatoxin were 860 and 630 \( \mu \text{g/kg} \) after irradiation doses of 5 and 25 kGy, respectively, compared with the control treatment of 965 \( \mu \text{g/kg} \). The percentages of the reduction of aflatoxins achieved were 40.1 and 42.7% after a dose of irradiation of 25 kGy in T3 for the total and B1 aflatoxins, respectively. These results were in agreement with the results obtained by Prado et al. [32], who found that even at a dose of 30 kGy in peanuts, the percentage of reduction achieved was about 61% and the destruction rate was nearly stable after the dose of 15 kGy. Also, the results were in agreement with Farage et al. [33], who found that an 83% reduction of aflatoxin after a 20- kGy dose of \( \gamma \)-irradiation of yellow corn and peanuts was achieved. On the contrary, Aziz and Youssef [34] found that
the dose of 20 kGy was sufficient to destroy completely AFB1 in peanuts, yellow corn, and cottonseed meal.

Table 6. Effect of microwave heating on aflatoxin (%) in contaminated chick feed\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Total aflatoxin (%)</th>
<th>Aflatoxin B1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>100\textsuperscript{a}</td>
<td>100\textsuperscript{a}</td>
</tr>
<tr>
<td>2</td>
<td>99.5\textsuperscript{a}</td>
<td>98.6\textsuperscript{a}</td>
</tr>
<tr>
<td>4</td>
<td>96.4\textsuperscript{ab}</td>
<td>95.1\textsuperscript{ab}</td>
</tr>
<tr>
<td>8</td>
<td>92.9\textsuperscript{b}</td>
<td>90.3\textsuperscript{d}</td>
</tr>
<tr>
<td>10</td>
<td>78.8\textsuperscript{c}</td>
<td>78.4\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a-c}Means with the same superscript letter within a column are not significantly different (\(P > 0.05\)).

\textsuperscript{1}Values are mean of 3 replicates (\(n = 3\)).

\textsuperscript{2}Concentrations for controls were less than the detection limits of the enzyme-linked immunosorbent assay procedure.

The effect of heating of contaminated feeds in a microwave was found to be the least effective in degradation of aflatoxins (Tables 5 and 6). The concentrations were decreased from 965 to 760 and from 890 to 690 \(\mu\)g/kg in treatment T1 for total and B1 aflatoxins, respectively. This could be due to the resistance of most mycotoxins to heat [35]. Also, the results were in agreement with those of Samarajeewa et al. [13], who found that 200\textdegree C was required for degradation of 60\% of aflatoxins in olive oils. In this study, the percentages of aflatoxin reduction were found to range between 21.2 and 33.3\% and 22.5 and 32.3\% in the total and B1 aflatoxins, respectively. The lower efficacy of microwave heating in decontamination of aflatoxins could be due to the result of shorter heating time, which was 10 min, as well as the feed particle geometrical dimension, which influences the rate of heating through decreasing the penetration depth of the microwaves through the feed. This could be due to the aflatoxins of naturally contaminated feeds being embedded within the feed commodities, on the contrary to the feed artificially contaminated with aflatoxins that are not fully embeddedwithin
the commodities. Therefore, the natural aflatoxins might be less likely to be degraded with radiation treatments, because they are within the commodity protected from radiation versus being on the surface of feeds when artificially applied. This could be an explanation of why the results were not in conformance with those obtained by Midio et al. [36] that the microwave or conventional heating had no effect on aflatoxin concentrations.

CONCLUSIONS AND APPLICATIONS:

1. Various methods of decontamination are found to vary in their efficacy in toxin removal from feed products.
2. Solar decontamination appears to be an efficient and cheaper method to use compared with microwave heating or γ-irradiation, or both, but is likely to be impractical on a large commercial scale. Effective vitamin concentrations may also be negatively affected.
3. The cost of the decontamination process is very important in choosing the cheapest and the most effective method in aflatoxin removal from the contaminated feed products.

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إزالة سمية الأفلا في ألعاب تم تلوثها بسموم الفطرية

باستخدام أشعة الشمس وأشعة جاما وأنسخن باستخدام الميكرويف

جزاء الله صقر* الشواهية خليل** فطافطة عبدالرحمن

المخص

بحثت هذه الدراسة كفاءة إزالة سمية الأفلا في ألعاب الدواجن من خلال تعرّضها لأشعة الشمس وأشعة جاما باستخدام التسخين بالميكرويف. حيث تم دراستها في عينات من ألعاب تم تلوثها بسموم الأفلا، أظهرت النتائج بأن تحطيم السموم الفطرية باستخدام أشعة الشمس كان ذو دلالة معنوية عند مستوى α=0.05 حيث أدى إلى خفض في مستوى سمو الأفلا B1 والسموم الفطرية الكلية علاوة على ذلك كان هناك ارتباط معنوي بين المعالمة ومدة التعرض لأشعة الشمس. إن سمو الأفلا والسماوم الفطرية الكلية انخفض مستويهما عند تعريض الألعاب الملوثة لأشعة الشمس بمقدار 43.3% و 39.9% و 75.5% و 65.9% عند تعريض الألعاب للمعالمة T1 لمدة 3 و 30 ساعة.

بينما الألعاب التي تم تعرّضها لأشعة جاما وتلك التي تم تسخينها بالميكرويف أدت إلى تأثير ذو دلالة معنوية في خفض مستوى السماوم B1 بـ 42.71% و 32.3% عند تعريضها لأشعة جاما وتسخينها بالميكرويف على التوالي للمعالمة T3 (استخدام 25 كيلوجرامي ولمدة 10 دقائق في الميكرويف). و عليه فان استخدام أشعة الشمس كان أكثر فاعلية في خفض مستوى سمو الأفلا B1 عند مقارنتها بأشعة جاما والتسخين بالميكرويف.

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** قسم الانتاج الحيوي، الجامعة الأردنية، عمان الأردن
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ABSTRACT

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Key words: aflatoxin, microwave, sunlight, γ-radiation, feed