Occurrence of Aflatoxins and Ochratoxin A in Indian Poultry Feeds

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ABSTRACT

From 1998 to 2001, 216 ingredients intended for incorporation into chicken feed, which included groundnut cake, maize, millets, rice bran, sorghum, soybean, sunflower, and mixed feeds, were assayed for aflatoxins and ochratoxin A contamination using an indirect competitive enzyme-linked immunosorbent assay. Thirty-eight percent of the samples were contaminated with aflatoxins and 6% with ochratoxin A. The incidence scores of aflatoxin contamination in excess of 10 μg/kg were 41 of 95 for maize, 18 of 30 for mixed feeds, 10 of 37 for groundnut, 6 of 29 for sorghum, 5 of 10 for sunflower, 3 of 14 for rice bran, and 1 of 8 for millet. Ochratoxin A contamination, in excess of 10 μg/kg, was found in 9 of 29 sorghum samples, 1 of 27 groundnut samples, 1 of 14 rice bran samples, 1 of 10 sunflower samples, and 2 of 8 millet samples. Ochratoxin A was not found in maize and mixed feeds. None of the three soybean samples contained ochratoxin A. This is the first report, to our knowledge, of co-occurrence of aflatoxins and ochratoxin A in Indian poultry feeds. The results confirm the importance of analysis of ingredients before incorporating them into mixed feeds.

The sudden appearance of turkey-X disease in 1960, which resulted in the death of more than 100,000 turkeys in the United Kingdom, led to the discovery of aflatoxins, a group of mycotoxins (1,3). Mycotoxins are highly toxic fungal secondary metabolites that occur in a wide variety of agricultural commodities. They are carcinogenic, mutagenic, teratogenic, nephrotoxic, and immunosuppressive agents. Mycotoxin contamination of agricultural commodities attracted worldwide attention because of the significant economic losses associated with their effects on human health, poultry, and livestock (7, 9, 11, 12). Although the aflatoxins that are produced by Aspergillus flavus and Aspergillus parasiticus are the most commonly known mycotoxins in poultry feeds, a number of other mycotoxins can act synergistically with them and thus can cause adverse effects to the poultry birds (2,6). For example, ochratoxin A produced by Aspergillus ochraceus and Penicillium verruculosum is toxic to poultry and in combination with other toxins can cause severe effects (6). Aflatoxins and ochratoxin A are immunosuppressive and generally affect the liver (hepatotoxic) or the kidney (nephrotoxic) and thereby give rise to a number of synergistic effects (5).

In most developing countries, limited or no facilities exist for monitoring feeds for mycotoxin contamination, which puts them at risk for health problems and major economic setbacks. Chemical methods for monitoring mycotoxins in feeds, e.g., thin-layer chromatography, liquid chromatography, gas chromatography, or mass spectrometry, are time-consuming, require extensive sample clean-up, and are expensive. Enzyme-linked immunosorbent assays (ELISAs) based on antigen-antibody reactions are now proving to be fast, specific, sensitive, and inexpensive methods for mycotoxin analysis (8).

To our knowledge, there are no reports in India on the co-occurrence of these two toxins in poultry feed ingredients. In the present study, an indirect competitive ELISA was used to survey aflatoxins and ochratoxin A in some of the most commonly used ingredients in poultry feeds.

MATERIALS AND METHODS

Materials. Aflatoxin B1, aflatoxin B1–bovine serum albumin (BSA) conjugate, ochratoxin A, ochratoxin A–BSA conjugate, BSA, and goat anti-rabbit immunoglobulin G–alkaline phosphatase conjugate were obtained from Sigma Chemical Co., Poole, England. Microtiter plates (Maxi-sorp) were obtained from Nunc, Denmark. All other reagents were of the highest analytical grade available. The polyclonal antibodies to aflatoxins and ochratoxin A used in these studies were produced in our laboratory (10,13,14). Antibodies produced for aflatoxin B1 have cross reactivities of 10, 4, and 0.2% to aflatoxin B2, G1, and G2, respectively, whereas antibodies raised for ochratoxin A were found to be specific for ochratoxin A.

Collection of samples. Feed samples that included groundnut cake, maize, millets, mixed feeds, rice bran, sorghum, soybean, and sunflower were collected from a feed manufacturing company.

Preparation of samples for ELISA. Each sample (1 kg) was thoroughly mixed and ground to a fine powder, and 20 g was processed for ELISA. Each 20-g quantity (in finely ground form)
TABLE 1. Incidence and range of aflatoxin (AF) and ochratoxin A (OA) in poultry feed samples as determined by indirect competitive ELISA

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Incidence, No. (%)</th>
<th>No. of samples with AF and OA (μg/kg) contents in the ranges of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AF</td>
<td>OA</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>10/27 (37)</td>
<td>1/27 (50)</td>
</tr>
<tr>
<td>Maize</td>
<td>41/95 (43)</td>
<td>0/95 (0)</td>
</tr>
<tr>
<td>Millets</td>
<td>1/8 (12)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Mixed feeds</td>
<td>18/30 (60)</td>
<td>0/30 (0)</td>
</tr>
<tr>
<td>Rice bran</td>
<td>1/14 (21)</td>
<td>1/14 (7)</td>
</tr>
<tr>
<td>Sorghum</td>
<td>6/29 (20)</td>
<td>9/29 (31)</td>
</tr>
<tr>
<td>Soybean</td>
<td>0/3 (0)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Sunflower</td>
<td>5/10 (50)</td>
<td>1/10 (10)</td>
</tr>
</tbody>
</table>

* See text for details.

was extracted in 100 ml of a mixture of methanol-water and KCl (70:30:0.5%) by blending in a Waring blender followed by shaking for 30 min. The extract was filtered through Whatman No. 41 filter paper and diluted to give 10-fold dilution in 0.2% BSA in phosphate-buffered saline containing 0.05% Tween 20 (PBS-T BSA) for processing by ELISA.

**Indirect competitive ELISA procedure for processing the samples.** The indirect ELISA procedure used was similar to that described by Thirumala-Devi et al. for aflatoxins (14) and ochratoxin A (13). Microtiter plate wells were coated with 0.1 μg/ml of aflatoxin B1–BSA or 1 μg/ml of ochratoxin A–BSA in 0.2 M sodium carbonate buffer, pH 9.6 (150 μl/well) (4), and incubated overnight at 4°C. Subsequent steps were performed at 37°C for 1 h followed by three washes with PBS-T. Antisera (1:80,000 for aflatoxin B1 and 1:100,000 for ochratoxin) were diluted in PBS-T BSA and held for 45 min at 37°C. Toxin standards (100 μl/well) at concentrations ranging from 100 ng/ml to 100 pg/ml were prepared in feed extracts (diluted 1:10) from samples that were free from the above toxins. Test samples were also diluted 1:10 in PBS-T BSA and added to the wells in 100-μl volume. All the samples were duplicated in two wells. Antiserum dilutions in a 50-μl volume were added to the standards and test sample extracts. Goat anti-rabbit immunoglobulin conjugated to alkaline phosphatase was used at a dilution of 1:2,000 to detect rabbit antibodies attached to aflatoxins or ochratoxin A. p-Nitrophenyl phosphate was used as a substrate at 1 mg/ml and allowed to develop for 1 h at room temperature. Absorbance was recorded at 405 nm (A405) with an ELISA plate reader (Titretek Multiskan, Lab Systems, Finland). Standard curves were obtained by plotting log_{10} values of toxin standards against optical density at A405. Concentration of toxin in the sample extract was determined from the standard curves and expressed in μg/kg using the following formula: toxin concentration (ng/ml) in sample extract × dilution with buffer × extraction solvent volume used (ml)/sample weight (g).

**RESULTS AND DISCUSSION**

Analysis of poultry feed ingredients obtained from a commercial feed manufacturing company showed that aflatoxins and ochratoxin A contamination occurred at high levels in groundnut cake, maize, sorghum, and millet samples (Table 1). Aflatoxins in the range of 10 to 3,500 μg/kg were found in groundnut cake and sorghum samples. Contaminations ranged from 10 to 1,500 μg/kg in mixed feeds, 10 to 300 μg/kg in maize, and 10 to 100 μg/kg in rice bran. High concentration of ochratoxin A was found in sorghum (400 μg/kg) and millet samples (145 μg/kg). Sunflower seeds were found to be contaminated with both aflatoxins and ochratoxin A in the range of 30 to 50 μg/kg. Ochratoxin A was not detected in maize samples or mixed feeds. Soybean was free from both the toxins. The study highlights the co-occurrence of aflatoxins and ochratoxin A at concentrations higher than 10 μg/kg in one groundnut cake sample, one rice bran sample, and eight sorghum samples.

Among the food industries, poultry is one of the most dynamic sectors. Recently, a tremendous growth has taken place in the feed industry because of new lucrative markets, especially for broilers. Nearly all feed samples are known to contain molds or mold spores, usually in small amounts. A number of factors, such as temperature, humidity, moisture, handling, and conditions during storage, influence the growth of molds and the production of mycotoxins by them. Moreover, the conditions in the tropical countries are favorable for the growth of molds and thus toxin production. Aflatoxins and ochratoxin A are proven to cause severe adverse effects and death of the poultry birds (2, 6, 11, 12). We have intensified our research efforts on these toxins after a major event in Andhra Pradesh. Aflatoxin-contaminated groundnut cake contributed to death of more than 200,000 broiler chickens in 1994. Recently two poultry farms in Chitradurgh, Karnataka State, in India lost more than 2,000 baby chickens as a result of feeding them aflatoxin-contaminated maize meal (unpublished data).

There have been attempts to switch to safer feeds in the poultry industry. To conduct surveys for the occurrence of these toxins in the feeds, it is essential to develop cost-effective and rapid methods for the quantitative estimation. We have chosen to apply immunochemical methods because they are rapid and do not require extensive sample clean-up. They are cost-effective and relatively easy to adapt to situations in developing countries. The enzyme immunoassays used in this study are suitable for aflatoxins and ochratoxin A detection up to 0.1 ng/ml in contaminated samples, with recovery rates of more than 90%, and they...
allow large numbers of samples to be analyzed under identical conditions. The procedures are relatively simple and robust, and the cost per sample is low. Thus, the technology can be applied to prevent highly contaminated commodities from entering into the feed chains and to facilitate reduction in the toxin content in the feed samples to less than 10 μg/kg by diluting the feed ingredients.

This study highlights the occurrence of aflatoxins and ochratoxin A at much higher levels (3,300 μg/kg) than the permissible level of 30 μg/kg in India. To overcome this problem, it is essential to take preventive measures to exclude or at least minimize mycotoxin contamination in poultry rations. Detection of toxins and rejection of contaminated ingredients are the most effective preventive strategies against aflatoxicosis and ochratoxicosis. This can be accomplished by routine monitoring for the presence of mycotoxins and through evolution of policies that discourage the marketing of toxin-contaminated feeds.

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REFERENCES