E. HANAK, E. BOUTRIF, P. FABRE, M. PINEIRO, (Scientific Editors), 2002. Food Safety Management in Developing Countries. Proceedings of the International Workshop, CIRAD-FAO, 11-13 December 2000, Montpellier, France, CIRAD-FAO. CIRAD CD-ROM, Montpellier, France.

Estimation of Aflatoxin Levels in Selected Foods and Feeds in India

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Abstract
Public and industrial interest in mycotoxin contamination has grown in India following significant deaths of poultry fed with contaminated feed in the 1990s. Using low-cost immunochemical tests, the research team analyzed samples of spices available at local markets and of ingredients used for industrial production of chicken feed. Aflatoxin contamination was particularly high for low-grade chillies (mainly purchased by the poor) and chilli powder sold as superior grade in supermarkets. High levels of contamination also occurred in maize and groundnut used for feed, a result which has prompted the feed industry to use the developed technologies for the routine testing of various ingredients. Monitoring of hazardous foods and feeds needs to take place more generally, and the marketing of contaminated products discouraged, to limit human and animal health risks.

Introduction

Currently in South Asia there are about 300 million poor people and nearly 70% of them live in rural areas. In recent years, overall food energy intakes per capita have risen but child malnutrition continues to be a problem (PINSTRUP-ANDERSEN et al., 2000). An overwhelming majority of agricultural commodities are vulnerable to contamination by mycotoxins. A number of mycotoxins have been identified and their effects on human and livestock health are known (BUSBY and WOGAN, 1979; BOSCH and PEERS, 1991; KATIYAR et al. 2000). The little that has been published indicates that there is significant exposure of the general population, including children, to various mycotoxins. In this paper, we deal with aflatoxin contamination of various commodities, which are likely to enter into the food and feed chain.

Surveys for the Occurrence of Aflatoxin in Selected Cereals and Spices

In order to conduct surveys for the occurrence of aflatoxins, it is essential to develop cost-effective and rapid methods for their quantitative estimation. ICRISAT has been investigating the problem of aflatoxin contamination for over 20 years. 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. We have intensified our research efforts on aflatoxins after a major event in Andhra Pradesh. Aflatoxin-contaminated groundnut cake contributed to the death of more than 200 000 broiler chickens in 1994. Recently, a poultry farm in Chitradurgh, Karnataka State, lost more than 2 000 baby chickens as a result of feeding them with aflatoxin-contaminated maize meal. In addition to maize and groundnut, many commodities including spices (JELINEK et al., 1989; VASANTHI and BHAT, 1998) are contaminated by aflatoxins. In this study we chose to analyze the ingredients of chicken feed used by one of the largest chicken feed manufacturers in India (Janaki Feeds), and to monitor commercially available spices for aflatoxin content.

Commercial kits to estimate aflatoxins by immunological methods are expensive, and there are problems associated with their importation. Therefore, efforts were made to produce at ICRISAT the good quality antisera that are a basic requirement for ELISA. High titered polyclonal antibodies were produced for aflatoxins by injecting the toxin hapten, conjugated to bovine serum album (BSA), at multiple sites into rabbits. A modified immunization schedule that involved several subcutaneous injections at multiple sites followed by a non-immunization rest period of six to eight months and a booster subcutaneous injection after this rest period resulted in the production of good quality antisera. Polyclonal antibodies produced for aflatoxin B1 recognized all the four aflatoxins B_1, B_2, G_1 and G_2.

A direct competitive ELISA was developed following the conjugation of aflatoxin-BSA to the reporter enzymes, alkaline phosphatase, horse radish peroxidase and penicillinase. Utilizing the penicillinase system it was possible to estimate aflatoxin content for less than one US dollar per sample.

Twenty five samples of each of ginger rhizomes, turmeric powder and rhizomes, black pepper seed and coriander seed obtained from local markets were analyzed. The results are summarized in Table I.

Chilli pod samples were collected randomly from regulated market yards from the major chilli-growing region in India, Guntur District. Chillies are marketed in three grades: grade-1 comprising largely reddish colored pods 8 to 10 cm in length without any visual damage or infection from fungal or insect attack; grade-2 with pods 6 to 8 cm in length and up to 20% discolored pods; grade-3 with pods 2 to 5 cm in length, more than 40% of which discolored, with apparent fungal growth. Also, “Ready to Use” chilli powders which are sold as “Superior Quality” were purchased from supermarkets. Aflatoxin contents were estimated using a modified indirect ELISA procedure. Of the 139 pod samples tested, 65% were contaminated with aflatoxin B_1 and 20% contained the toxin at higher than 30 μg per kg (Table II). In all the samples, only aflatoxin B_1 was detected. The highest concentration of 969 μg/kg was detected in one sample of grade-3 chillies. Nearly forty percent of the chilli powders sold in supermarkets contained aflatoxins and nine percent of them contained aflatoxin levels higher than 30 μg/kg (REDDY et al., in press). This finding highlights the risk populations are exposed to by consuming low grade chilli samples as well as powders that, unfortunately, are marketed under “Superior Grade” label.

Recently a tremendous growth has taken place in the feed industry because of new lucrative markets, especially for broilers. Various ingredients of poultry feed were obtained from Janaki Feeds and tested for aflatoxin content. More than 200 samples of groundnut cake, maize, millets, mixed feeds, rice bran, sorghum, soybean and sunflower were tested (Table III). Aflatoxin contamination occurred at high levels (3300 μg/kg) in maize and groundnut. Soybean samples were found to be free from aflatoxins. This result prompted the feed industry to use our technologies for the routine testing of various feed ingredients for aflatoxin levels. Currently two private sector companies and one public sector organization are using our technology.

Recently, we also received additional funding from the UK's Department for International Development (DFID) for a project, which should ultimately lead to the production of aflatoxin-free groundnuts. We will be investigating factors that influence aflatoxin contamination at or before harvest, during post-harvest handling and in post-harvest storage. We have also recently produced antibodies for aflatoxin M1 (K. THIRUMALA DEVI, unpublished). This became essential because farmers use groundnut haulms containing small pods as cattle feed. Often these pods are contaminated with aflatoxin and this results in losses in milk yield and in contamination with aflatoxin M_1 of milk obtained from these cattle. Cattle fed with contaminated pods show signs of diarrhea and ephemeral fever.

Conclusion

It is apparent from this study, that grade 3 chilli pods, chilli powders sold in supermarkets and maize and groundnut samples employed in preparing poultry feed can harbor aflatoxin at non-permissible levels (>30 μg per kg). In order to overcome this problem, it is essential to monitor these commodities periodically and evolve policies which discourage the marketing of toxin-contaminated foods and feeds.

References

BOSCH, F.X., PEERS, F., 1991. Aflatoxins: data on human carcinogenic risk. In relevance to human cancer of N-Nitroso compounds. *In:* Tobacco and Mycotoxins, ed. J.K. O'NEILL, J. CHEN, H. BARSTCH, pp. 48-53. International Agency for Research on Cancer, Lyon, France.

BUSBY, W.F. Jr., WOGAN, G.N., 1979. Food-borne mycotoxins and alimentary mycotoxicoses. *In:* Food-borne infections and Intoxications, 2nd ed., H. RIEMANN, F.L. BRYAN, pp. 519-610. Academic Press, New York.

JELINEK, C.F., POHLAND, A.E., WOOD, G., 1989. Worldwide occurrence of mycotoxins in foods and feeds. Journal of the Association of Official Analytical Chemist International, 72:223-230.

KATIYAR, S., THAKUR, V., GUPTA, R.C., SARIN, S.K., and DAS, B.C., 2000. P_{53} tumor suppressor gene mutations in hepatocellular carcinoma patients in India. Cancer, 88:1565-73.

PINSTRUP-ANDERSEN,P., PANDYA-LORCH, R.C., ROSEGRANT, M.W., 2000. Food Security: Problems, Prospects, and Policies. 2020 Vision Discussion Paper. Washington DC: International Food Policy Research Institute.

REDDY, S.V., KIRAN MAYI, D., UMA REDDY, M., THIRUMALA-DEVI, K., REDDY, D.V.R., in press. Aflatoxins in different grades of chillies (*Capsicum annum* L.) in India as determined by indirect competitive ELISA. Food Additives and Contaminants.

ASANTHI, S., BHAT, R.V., 1998. Mycotoxins in foods – occurrence, health and economic significance and food control measures. Indian Journal of Medical Research, 108:212-224.

Table I. Aflatoxin content of selected spice samples[1].

Commodity[2]	No. containing toxin/total no. analysed	Aflatoxin content[3] (range)
Ginger rhizomes	2/25	24-26
Turmeric	0/26	-
Black pepper	2/28	16-20
Coriander	0/50	-

1. THIRUMALA et al., unpublished.
2. All samples obtained from local markets.
3. Analyzed by an indirect-competitive ELISA.

Table II. Aflatoxin levels in different grades of chilli samples analyzed by indirect competitive-ELISA.

Sample	Numbers analyzed	No. of samples with aflatoxin in the range (μg/kg) 0	<10	11-30	31-50	51-100	>100
Chilli grade 1	42	21	16	3	1	1	0
Chilli grade 2	38	13	10	6	3	4	2
Chilli grade 3	44	3	21	4	3	2	11
Cold store	15	12	2	0	1	0	0
Chilli powders	43	26	12	1	0	3	1

Table III. Incidence and range of aflatoxin in poultry feed samples as determined by indirect-competitive ELISA.

Percent samples containing toxin/ No. of samples analyzed		Aflatoxin range (μg per kg) 10-29	30-49	50-100	>100
Groundnut cake	10/27	2	1	3	4
Maize	41/95	15	9	8	9
Millets	1/8	1	0	0	0
Mixed feeds	18/30	11	3	1	3
Rice bran	3/14	1	0	2	0
Sorghum	6/29	2	2	0	2
Soybean	0/3	0	0	0	0
Sunflower	5/10	0	5	0	0