Detection of Aflatoxin B_1 in Imported Maize Kernel Used as Feed by Enzyme-Linked Immunosorbent Assay

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(Received 30 October 1990/Accepted 16 May 1991)

J. Vet. Med. Sci. 53(4): 767–768, 1991

KEY WORDS: aflatoxin B_1, ELISA, feed.

Aflatoxins are toxic secondary metabolites produced by *Aspergillus (A) flavus* and *A. parasiticus* [4]. Aflatoxin B_1 (AFB_1), the most toxic compound in this series, has been found to be one of the most potent carcinogens occurring naturally [4]. Frequent contamination with AFB_1 of agricultural commodities such as peanuts, corn, and animal feedstuffs, has become a potential hazard to human and animal health [4]. In Thailand and Indonesia, the contamination of agricultural products with aflatoxins is a serious economic problem [1, 3, 5].

In Japan, almost all of the maize kernel is imported and is usually used as feed, though the contamination of imported maize with AFB_1 has been suspected. It is prohibited to use feeds containing over 20 ppb of AFB_1 for adult cattle and over 10 ppb of AFB_1 for young cattle as the maximum values (Office Memorandum of General Director of the Bureau of Livestock Industry, Ministry of Agriculture, Forestry and Fisheries). In general, AFB_1 has been detected by high performance liquid chromatography (HPLC) and thin layer chromatography. However, the sensitivity of HPLC for the detection of AFB_1 is not very high [6].

Recently, Ueno [8] reported that enzyme-linked immunosorbent assay (ELISA) can be applied for the detection of AFB_1. We also showed that ELISA is useful for the detection of AFB_1 in food products. Namely, the values obtained by ELISA correlated with those by HPLC and the sensitivity of ELISA was higher than that of HPLC [2]. The detection limit of ELISA for AFB_1 was 1 pg/assay and the recovery rate of AFB_1 from maize exceeded 80%. Using ELISA, we found that food products were broadly contaminated with AFB_1, though its content was less than 10 ppb of sample.

In the present experiments, we attempted to detect AFB_1 in imported maize kernel as feed by ELISA because the product was also suspected to be contaminated with AFB_1.

Fourteen samples of maize imported from the United States of America, People's Republic of China, and Thailand in 1984, were used for the detection of AFB_1. These samples were supplied by the National Inspection for Agricultural Fertilizer and Feed. Those imported samples were taken in feed companies according to the Law Concerning Safety Assurance and Quality Improvement of Feed, and stored at −20°C.

Table 1. Content of aflatoxin B_1 in maize imported as feed

Imported from	Sample No.	Aflatoxin B_1 detected ng/g
United States of America	1	4.30±0.52[a]
	2	0.49±0.21
	3	1.91±0.32
	4	2.66±0.36
	5	ND[b]
	6	4.18±0.42
	7	4.12±0.42
	8	ND
	9	ND
	10	4.21±0.41
People's Republic of China	1	1.82±0.31
	2	7.27±0.31
Thailand	1	23.27±1.21
	2	31.21±1.65

a) Mean±standard deviation.
b) ND: Not detectable.
ELISA was repeated three times in an extracted sample.

Extraction and cleanup procedures of AFB_1 from homogenized maize samples were carried out according to the method of Minamisawa *et al.* [7]. ELISA was carried out as previously described [2], and was repeated three times in an extracted sample and the values obtained by ELISA were expressed as mean ± standard deviation (SD) (Table 1). The results showed that the values were highly reproducible. In two samples of maize imported from Thailand, over 20 ng of AFB_1 per gram of maize was detected (23.27±1.21 ng and 31.21±1.65 ng, respectively). AFB_1 was also detected in seven samples imported from the U.S.A. and in two samples from the People's Republic of China and the content was less than 10 ng/g. Eventually, 11 out of the 14 imported maize samples were found to be contaminated with AFB_1. Particularly, it is noteworthy that 2 out of the 2 samples from Thailand are highly contaminated with AFB_1. Presently maize kernel is still imported from Thailand but not as frequently as previously. Contamination with AFB_1, which is a highly carcinogenic mycotoxin, should be checked before maize is imported to Japan.

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