CORN IS A MAJOR SOURCE OF MYCOTOXINS IN POULTRY FEED AT THE TIME OF FORMULATION

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ABSTRACT

Every truckload of corn (n = 52) entering and every batch of poultry feed (n = 290) leaving a Bogor feedmill over one year was analysed for aflatoxins, zearalenone, ochratoxin A and sterigmatocystin. All but 2 lots of corn and 274 of the batches of chicken feed contained aflatoxins. Zearalenone was detected in 11 corn samples but was not found in the formulated feed. Ochratoxin A was detected in one corn sample, but not in feed. Corn can account for all of the aflatoxin in the feed since levels were always lower in the finished product.

INTRODUCTION

Mycotoxins, toxic metabolites of fungi, cause a variety of diseases in poultry. Mycotoxins are best known for their primary toxicity - acute or chronic toxicity directly related to the mycotoxin. For example, aflatoxins at high doses are acutely hepatotoxic while lower doses over a prolonged period may cause hepatic carcinoma. The secondary toxic effects of mycotoxins are those indirectly related to exposure to mycotoxins, such as an increase in infectious disease due to suppression of the immune response. The secondary toxic effects of mycotoxins are particularly difficult to demonstrate, but they may be more important than primary toxicity (Smith, 1982).

Poultry feeds worldwide are frequently contaminated by aflatoxins and other mycotoxins (Funnell, 1979; Buckle, 1983; Dutton and Westlake, 1985). Previous reports from Indonesia indicate that more than 80% of local poultry feeds which are based on corn are contaminated with aflatoxins (Ginting, 1985; Ginting, 1986). Corn is a common source of mycotoxins around the world. Aflatoxins, ochratoxin A, moniliformin, cyclopiazonic acid, zearalenone and other fusarial toxins have been found. Aflatoxin has been reported in corn in Indonesia and corn was thus suspected to be a major source of aflatoxin and perhaps other mycotoxins in poultry feed at the point of manufacture.

The present study was undertaken for several purposes: firstly to determine the correlation between mycotoxins contaminating corn and poultry feed over one year, as represented by one feed mill near Bogor, West Java; secondly, to determine the degree of variation in mycotoxin concentrations in chicken feed produced by this mill during the year; thirdly, to assess various rapid indicators of fungal damage of corn in relationship to mycotoxin contamination. These indicators are: bright greensh-yellow fluorescence (BGYF) under ultra violet light, associated with *Aspergillus* species (Fennel *et al.*, 1973); insect damage, which commonly facilitates fungal invasion; purple kernels, associated with *Fusarium* species (Blaney *et al.*, 1984); and obviously mouldy kernels. Preliminary results of this study have been presented (Ginting *et al.*, 1985; Widiastuti *et al.*, 1986).

MATERIALS AND METHODS

A sample (about 1 kg) was taken from every truckload of corn (n = 52) entering and every batch of chicken feed (n = 290) leaving a Bogor feedmill over the period of one year beginning in August 1985. The corn was said to have originated primarily from Lampung with some from Central Java, but no information was available on the time of corn harvest or period in storage. Before analysis for mycotoxins, each corn sample was separated into five subsamples of kernels: bright green-yellow fluorescent (BGYF) kernels under 365 nm ultraviolet light, insect-damaged kernels, purple kernels, mouldy kernels and good kernels. The weights of these subsamples were recorded.

Each subsample or corn kernels and each sample of chicken feed was milled through a 0.7 mm screen, extracted with organic solvents and the components separated by 2-dimensional thin layer chromatography (Blaney *et al.*, 1984). Aflatoxins B1, B2, G1, G2, ochratoxin A, sterigmatocystin and zearalenone can be readily detected by their fluorescence under ultraviolet light after separation by this technique. Mycotoxin concentrations were determined by visual comparison of fluorescent intensity of sample spots with standards, following suitable dilution.

Statistical procedures (Gill, 1971) were applied to the results of analyses of batches of chicken feed to check homogenity of monthly variances (Bartlett's test), differences between months (Box's F-test) and

to compare the group of wet season monthly means with the dry season means (Scheffe's procedure).

RESULTS AND DISCUSSION

Results of analysis of 52 lots of corn and 290 batches of chicken feed are summarised in Table 1. All but 2 lots of corn contained aflatoxins and 94.5% of the batches of chicken feed contained aflatoxin B1. Aflatoxin B1 was the predominant form, usually accompanied by lower levels of B2. Aflatoxins G1 and G2 respectively were found in 33% and 14% of the corn samples as well as in 7% and 2% of the feed samples. Both *Aspergillus flavus* and *A. parasiticus* are probably involved in aflatoxin contamination of Indonesian corn since *A. flavus* is usually reported to produce only B1 and B2, while *A. parasiticus* may produce G1 and G2 in addition to B1 and B2 (Dorner *et al.*, 1984).

Twenty-two percent of the chicken feeds had aflatoxin B1 levels of 0.1 ppm or higher. These results are of concern since levels of 0.1-0.2 ppm have been associated with subclinical toxicity in broiler chickens (Giambrone *et al.*, 1985). Corn can account for all of the aflatoxin in the formulated chicken feeds since levels were always lower in the finished product. Also, mean concentrations of aflatoxin B1 in feed over the year were 50% of mean concentrations in corn over the year, which corresponds to the usual incorporation level of corn into feeds (50-60%).

Zearalenone was detected in eleven (21%) corn samples but was not found in any of the formulated feeds, presumably due to dilution. Ochratoxin was found at 0.19 ppm in one subsample of mouldy kernels. Sterigmatocystin was not detected in any sample.

The separation of kernels into different types was done to assess the usefulness of abnormal kernels as markers of corn quality. The highest concentrations of aflatoxin were found in BGYF and mouldy kernels (Table 1). BGYF is associated with preharvest invasion of corn by *Aspergillus* species and can be a good indicator of aflatoxin contamination (Fennel *et al.*, 1973; Blaney, 1981). However, in the present study there was no obvious association between the proportion of BGYF and/or mouldy kernels and aflatoxin content of the composite sample. The presence of obviously mouldy kernels may indicate fungal growth during storage and this may have clouded any correlation with BGYF, which is an indication of pre-harvest fungal invasion. It remains possible that BGYF may be a good indicator of the potential for increased aflatoxin contamination of certain lots of corn in storage, if the test is applied immediately after harvest.

Although zearalenone was only found in purple kernels, such kernels were not good indicators of zearalenone contamination since most subsamples of purple kernels did not contain zearalenone. The purple discolouration of corn is most characteristic of *Fusarium graminearum* infection (Sutton, 1982; Blaney *et al.*, 1986), which leads to substantial zearalenone concentrations. However, *Fusarium moniliforme*, which produces only trace amounts of zearalenone, also causes a pink-purple colour. Consequently, the presence of *F. moniliforme* could cloud the correlation between purple kernels and zearalenone concentrations. Nevertheless, the absence of purple kernels may still be usefull as an indicator of the likely absence of zearalenone and perhaps other *Fusarium* toxins. The finding of zearalenone in a few samples of corn does indicate

Table 1. Mycotoxins (ppm) in corn and chicken feed

Sample	% of composite range	Concentration range	
		Aflatoxin B1	Zearalenone
Corn	2.1— 2.1	.3— 2.2	.3— 2.3
purple kernels	0.1— 6.3	.001— 2.0	.05—13.5
insect-damaged kernels	2.1—22.9	.001— 1.5	ND
BGYF kernels	0.1— 2.2	.010—20.0	.58
mouldy kernels	0.2—14.5	.016— 5.0	ND
good kernels	75.3—96.4	.001— 1.0	ND
composite	100	.001— 1.1	.001— .014
Chicken feed		.001— 0.50	ND

ND = not detected

infection by *Fusarium* species. Zearalenone was first reported in Indonesia in corn growing at high altitude by Widiastuti *et al.* (1985). Zearalenone is an estrogenic mycotoxin of greatest hazard to swine (Sundlof and Strickland, 1986) but it may also affect poultry (Bock *et al.*, 1986). More significantly, zearalenone is often accompanied by toxic trichothecenes, moniliformin and other as yet unidentified *Fusarium* toxins (Thiel *et al.*, 1982; Lee *et al.*, 1985). No attempt was made to assay for these other mycotoxins.

The monthly average levels of aflatoxin B1 (Table 2) are derived from about four analyses per month for corn and about 25 per month for the formulated feed. Since Bartlett's test showed that the variances between different months are not homogeneous, Box's F-test with approximate degrees of freedom was used and indicated that there are real differences between months. The feed levels of aflatoxin are significantly increased during the wet season months of December to May ($P < 0.01$) compared to the dry season months, according to Scheffe's procedure for comparing groups of means. For corn, variation within month probably obscures any seasonal difference. These results indicate that an increased risk of mycotoxicosis may occur during the wet season. Since levels are relatively low, the effect may only be evident as an increase of secondary mycotoxic problems in chickens, although these levels are toxic to ducks (Hetzel *et al.*, 1984). Hetzel *et al.* (1984) have reported very similar aflatoxin levels in duck layer rations with a peak near 0.1 ppm aflatoxin B1 during the 1980 wet season.

Table 2. Monthly average aflatoxin B1 levels in corn and chicken feed

Month	Aflatoxin B1 (ppm) in	
	Corn*	Chicken Feed
August 1985	.056	No sample
September	.008	.005
October	.007	.005
November	.010	.002
December	.130	.054
January 1986	.053	.040
February	.064	.052
March	.369	.095
April	.243	.220
May	.105	.080
June	.152	.035
July	.095	.030
August	.013	.011

* Composite sample

The very high frequency of aflatoxin contamination of chicken feed observed at this feed mill may not be representative of other mills nearby or throughout Indonesia and consequently more surveys are required. In addition, the possible changes in quality of chicken feed, from the time of manufacture to the point of consumption, should be investigated since transportation, storage and aspects of presentation of feed to poultry provide opportunities for mould growth and further mycotoxin production.

Of the many questions arising out of this study, two appear to be of greatest urgency to the development of the intensive poultry industry in Indonesia. Firstly, are there predictable major differences in the level of mycotoxin contamination of corn from different geographical regions or growing seasons? Secondly, can the mycotoxins in poultry feeds be linked to disease or production loss? Neither will be easy to answer.

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