Mycopathologia 104: 153–156 (1988)



# Cyclopiazonic acid in combination with aflatoxins, zearalenone and ochratoxin A in Indonesian corn

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Accepted 1 August 1988 in revised form

*Key words:* aflatoxin, corn, cyclopiazonic acid, Indonesia, mycotoxins

## Abstract

The mycotoxin, cyclopiazonic acid (CPA), was detected at concentrations as high as 9 ppm in 21 of 26 corn samples from a Bogor poultry feedmill. This is the first demonstration of the natural occurrence of CPA in Indonesia. CPA was always accompanied by other mycotoxins, especially aflatoxins, suggesting that the interactive toxicity of these mycotoxins to poultry should be investigated.

## Introduction

Interest in mycotoxins has grown rapidly since the first demonstration of aflatoxicosis in turkeys, turkey 'X' disease, in 1961 [1]. However, despite extensive research over several decades, new fungal toxins and their disease syndromes are still being characterized [18]. The extent of mycotoxin problems in Indonesia is largely unknown, but Indonesia has the environmental conditions – high rainfall and humidity – favourable for mould growth and toxin production.

Previous surveys of Indonesian livestock feeds and foodstuffs for human consumption have indicated a high incidence of contamination with aflatoxins, products of *Aspergillus flavus* and *A. parasiticus* [10, 11, 24, 35]. Peanut and corn products were the most commonly affected commodities. Zearalenone and ochratoxin A have also been detected in corn [35]. Another mycotoxin, cyclopiazonic acid (CPA), may be produced by aflatoxigenic *A. flavus* as well as by other *Aspergillus* spp. and *Penicillium* spp. that infect corn [6, 7, 9]. Consequently we assayed Indonesian corn for CPA and investigated the combination of CPA with other mycotoxins.

## Materials and methods

Twenty-six samples of corn remaining from a previous mycotoxin survey were used in this study [35]. The corn had been collected regularly during a one year period from a poultry feed mill near Bogor.

Each corn sample (about 1 kg) was separated into 5 subsamples: bright green-yellow fluorescent (BGYF) kernels under 365 nm ultra-violet light; insect-damaged kernels; purple kernels; mouldy kernels; and good kernels. Each subsample of kernels (25 g) was milled to pass through a 0.7 mm screen and then extracted using 102 ml of acetonitrile:4% KCl:5 M HCl (90:10:2) for 30 minutes for vigorous shaking (Gallenkamp flask shaker). The mixture was filtered and 50 ml of filtrate added to 50 ml of water prior to extraction 2× using 50 ml of hexane. The hexane phase was discarded and the acetonitrile phase was extracted 2× with 50 ml of methylene chloride. The upper aqueous phase was discarded and the methylene chloride-acetonitrile phase was passed through anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator.

The residue was redissolved in 20 μl of acetone and spotted on 5×5 cm silica gel thin layer chromatography (TLC) plates (E. Merck, Darmstadt, Art. 5553) for two-dimensional TLC [3]. The developing solvent for the first dimension was chloroform:acetone 9:1 (v/v) and for the second toluene:ethyl acetate:formic acid 5:4:1 (v/v/v). Aflatoxins, zearalenone and ochratoxin A were detected under ultraviolet light at Rf values identical to commerical standards (Sigma Chemical Co., St. Louis). CPA was detected as a violet-blue spot under visible light after spraying with p-dimethylaminobenzaldehyde solution (2 g in 50 ml of 95% methanol plus 50 ml of conc. HCl). Rf in the first dimension was 0.2 and in the second 0.7. CPA was positively identified by coincidence of migration with authentic CPA in several solvent systems during TLC, and by an identical colour reaction to authentic CPA after spraying with p-dimethylaminobenzaldehyde. Mycotoxins were quantified by visual comparison with standard solutions of the mycotoxins, following sequential dilutions if necessary. Authentic CPA was obtained from Dr R. J. Cole, United States Department of Agriculture. Aflatoxins, ochratoxin A and zearalenone were confirmed

*Table 1.* Mycotoxins (ppm) in corn.

| Sampling date | CPA | Aflatoxin | | | | | |
|---|---|---|---|---|---|---|---|
| | | B1 | B2 | G1 | G2 | ZEA | OA |
| 28.09.85 | 1.17 | 0.028 | 0.018 | 0.007 | tr | 0.014 | ND |
| 02.11.85 | 3.13 | 0.026 | 0.002 | ND | ND | ND | ND |
| 09.11.85 | ND | 0.002 | tr | ND | ND | ND | ND |
| 16.11.85 | 0.12 | 0.018 | 0.008 | ND | ND | ND | ND |
| 23.11.85 | ND | tr | ND | tr | tr | ND | ND |
| 30.11.85 | ND | 0.003 | ND | ND | ND | ND | ND |
| 07.12.85 | 0.57 | 0.228 | 0.158 | ND | ND | 0.008 | ND |
| 25.01.86 | 0.30 | 0.143 | 0.011 | ND | ND | ND | ND |
| 01.02.86 | 5.50 | 0.086 | 0.002 | ND | ND | ND | ND |
| 08.02.86 | 0.78 | 0.150 | 0.007 | 0.003 | ND | ND | ND |
| 22.02.86 | 0.15 | 0.047 | tr | 0.001 | ND | ND | ND |
| 15.03.86 | 0.24 | 0.121 | 0.010 | 0.010 | ND | ND | ND |
| 22.03.86 | 1.69 | 1.05 | 0.014 | 0.001 | ND | 0.008 | ND |
| 03.05.86 | 1.56 | 0.141 | 0.007 | 0.002 | ND | 0.007 | ND |
| 17.05.86 | 9.22 | 0.087 | 0.018 | ND | ND | ND | ND |
| 07.06.86 | 0.03 | 0.031 | ND | ND | ND | ND | ND |
| 21.06.86 | 0.28 | 0.068 | 0.004 | ND | ND | ND | ND |
| 28.06.86 | ND | 0.458 | 0.012 | ND | ND | ND | ND |
| 15.07.86 | 1.04 | 0.003 | ND | ND | ND | ND | 0.003 |
| 22.07.86 | 0.16 | 0.160 | ND | ND | ND | ND | ND |
| 03.08.86 | ND | ND | ND | ND | ND | ND | ND |
| 10.08.86 | 0.15 | 0.002 | tr | tr | ND | ND | ND |
| 15.08.86 | 3.47 | 0.012 | tr | ND | ND | ND | ND |
| 23.08.86 | 0.19 | 0.015 | 0.001 | ND | ND | 0.004 | ND |
| 30.08.86 | 2.39 | 0.047 | tr | 0.001 | 0.001 | 0.001 | ND |
| 06.09.86 | 8.08 | 0.050 | 0.001 | tr | ND | 0.002 | ND |
| incidence (%) | 81 | 96 | 77 | 38 | 12 | 31 | 4 |

Abbreviations: CPA = cyclopiazonic acid; ZEA = zearalenone; OA = ochratoxin A; ND = not detected; tr = trace (<0.001 ppm) as reported previously [35]; CPA could be extracted into dilute sodium bicarbonate solution and back into chloroform after reacidification with hydrochloric acid, as an additional confirmatory test.

## Results

CPA was found in 21 of the 26 corn samples and was always accompanied by aflatoxins (Table 1). Half of the CPA-contaminated samples had levels of >1 ppm. CPA was found in all types of subsamples of kernels with approximately equal frequency, but the highest levels were found in insect-damaged and BGYF kernels. Zearalenone was detected in seven samples and ochratoxin A in one.

## Discussion

This is the first report of the occurrence of CPA in Indonesia. Although there are a number of recent references on methodology for CPA, including colorimetric [28], liquid chromatographic [14] and thin layer chromatographic methods [15], reports on occurrence of CPA in agricultural products are rare. CPA is known to occur naturally in corn [9], peanuts [16], kodo millet seed [27] and cheese [17]. In the first report of natural occurrence, Gallagher *et al.* [9] found CPA in 6 aflatoxin-contaminated North American corn samples. On the other hand, Dutton and Westlake [8] found no CPA in 800 samples of South African agricultural commodities, including 155 corn samples, of which 25% contained aflatoxin. We could find no other reports regarding the natural occurrence of CPA in corn.

CPA may be produced by several *Aspergillus* species and *Penicillium* species [33]. Bright green-yellow fluorescence is associated with preharvest infection of corn with *Aspergillus* species [2]. Similarly, insects feeding on developing corn ears have been associated with increased fungal invasion [4, 32]. Studies on aflatoxigenic and non-aflatoxigenic strains of *A. flavus* have shown that strains may produce aflatoxin alone, CPA alone, aflatoxin plus CPA or neither toxin [9]. Of the aflatoxigenic fungi, *A. parasiticus* can produce aflatoxins B1, B2, G1 and G2 but not CPA, whereas *A. flavus* can produce CPA and aflatoxins B1 and B2, but does not usually produce G1 and G2 [7]. Our results indicate that all 4 aflatoxins can occur with CPA.

CPA has been implicated in two natural outbreaks of disease. Seeds of kodo millet (*Paspalum scobriculatum*) that caused 'kodua poisoning' of man were found to contain CPA (amount not stated). *Aspergillus flavus* and *A. tamarii*, both CPA producers, were isolated from toxic seed [27]. In the second case, it has been postulated, on the basis of distinctive clinical signs, that CPA was involved in the classical turkey 'X' disease syndrome [5]. Although no natural disease of domestic animals has been conclusively related to CPA, toxicity has been induced experimentally in chickens [6], swine [21], dogs [25], rats [12, 22, 26, 34] and guinea pigs [29].

Although several investigators have stressed that CPA is usually accompanied by aflatoxins and that the possibility of synergistic toxicity is very real, one study on the combined effects in rats after subacute exposure revealed no synergistic toxicity [23]. Nevertheless synergistic toxicity has been demonstrated for several mycotoxins [13, 19, 30] and the interaction of CPA and aflatoxins in poultry warrants investigation. Similarly, studies cited by Stoltz [31] suggest that feed naturally contaminated with a mycotoxin is likely to be more toxic than feed artificially contaminated with the same level of pure toxin. Further work is required to investigate the toxicity for man and livestock of Indonesian corn naturally contaminated with CPA and other mycotoxins, especially aflatoxins, and to examine the effects on co-contaminants of treatments specifically designed to destroy aflatoxins.

## Acknowledgements

The authors wish to thank the feedmill for cooperation and support during this study and Dr R. J. Cole, USDA, for providing authentic CPA.

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