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Occurrence of the Mycotoxin Cyclopiazonic Acid in Meat after Oral Administration to Chickens

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Four-week old chickens were administered 0.5, 5.0, or 10.0 mg of cyclopiazonic acid (CPA)/kg of body weight by crop intubation and were killed 3, 24, 48, or 96 h later. Breast and thigh muscle was removed from the carcasses, and the content of CPA in the meat was measured by a high-performance liquid chromatographic procedure. CPA was found in the meat from birds in each dose group, and the amount found was dependent on both dose given and the elapsed time after dosing. The maximum muscle content of CPA occurred 3 h after administration and ranged from 50 ppb in birds given 0.5 mg/kg to over 5000 ppb in some birds given 10.0 mg/kg. Birds given the highest dose of CPA eliminated CPA from meat at a slower rate than other treatment groups.

The fungal metabolite cyclopiazonic acid (CPA; Figure 1) is a toxic indole tetramic acid first isolated from *Penicillium cyclopium* Westling (Holzapfel, 1968). The toxicity of CPA to rats was demonstrated by Purchase (1971) and has since been shown by others (van Rensburg, 1984; Morrissey et al., 1985). Toxicity occurs in a number of other species, including pigs (Lomax and Cole, 1983; Lomax et al., 1984), chickens (Dorner et al., 1983), mice (Nishie et al., 1985a), dogs (Neuhring et al., 1985), and rabbits (Nishie et al., 1984). CPA is also suspected of causing symptoms of "kodua poisoning" in humans that consumed kodo millet seed in India (Rao and Husain, 1985). The discovery that *Aspergillus flavus* isolates are capable of producing CPA (Luk et al., 1977; Gallagher et al., 1978) has caused increased concern over the potentially harmful effects of this toxin, since *A. flavus*, one of the fungi responsible for the production of the highly toxic and carcinogenic aflatoxins, is a frequent contaminant of major agricultural commodities. Instances of natural occurrences

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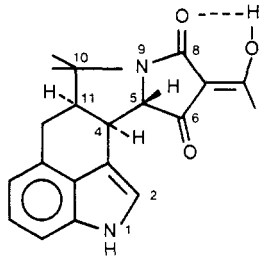

Figure 1. Structure of cyclopiazonic acid.

of CPA contamination have been reported in both corn (Gallagher et al., 1978) and peanuts (Lansden and Davidson, 1983). Other fungi, including industrially important species such as *Penicillium camemberti* and *Aspergillus oryzae*, have been shown to produce CPA (Cole, 1984).

Clinical signs of CPA intoxication include weight loss, weakness, inappetence, vomiting, diarrhea, dehydration, depression, opisthotonus, convulsions, and death. The neurological effects of CPA (Nishie et al., 1985a,b) are of particular interest, since some of the signs observed (opisthotonus, catalepsy, hypothermia, sedation) are reminiscent of those described by Blount (1961) for "Turkey X Disease". This disease killed 100 000 turkey poults in England and was later attributed to *A. flavus* contamination of peanut meal that had been incorporated into poultry rations. Aflatoxins were eventually isolated from the rations and are historically cited as the causative agent of Turkey X disease. However, the neurological symptoms observed are not generally associated with aflatoxicosis, and Cole (1986) has speculated that since both aflatoxin and CPA can be produced by *A. flavus*, both toxins may have been involved in the syndrome.

In a study of the distribution and excretion of radioactively labeled CPA in rats, we discovered that CPA apparently accumulated to a significant degree in skeletal muscle tissues (Norred et al., 1985). After either oral or intraperitoneal administration, 48% of the radioactive dose was accounted for in muscle 6 h after dosing. This finding raised concerns that similar accumulation in the meat of food animals could be a source of ingestion of this mycotoxin by humans if the animals were inadvertently fed CPA-contaminated rations. To assess the extent to which this could occur in poultry, we first developed an analytical method for CPA in meat (Norred et al., 1986) and then dosed chickens with varying amounts of CPA. The chickens were killed at intervals up to 96 h after dosing, and the meat from the birds was analyzed. The results of these investigations are described in the present report.

EXPERIMENTAL SECTION

Materials. Cyclopiazonic acid was produced using the fungus *Penicillium griseofulvum* (NRRL 3523) and purified as previously described (Dorner et al., 1983). The purity of the CPA was 99%, based on thin-layer chromatography and its UV extinction coefficient at 284 nm. Other chemicals were reagent grade, except solvents, which were pesticide or HPLC grade.

Animals. Unsexed White Leghorn chickens (200-day old) were purchased locally and provided grower diet (mash form) and water ad libitum. For the first week, heat lamps were used to supplement the room temperature in order to maintain a temperature of 30–35 °C. The diet was analyzed by the method described below, and no detectable levels of CPA were found. After 3 weeks, 140 healthy birds were randomly assigned to treatment groups, identified by leg bands, and housed five per cage in suspended stainless-steel cages. Ten birds were assigned to each CPA dose–kill time group, and five birds were used for each control–kill time group. At 4 weeks of age, the birds were dosed by crop gavage with 0.5, 5.0, or 10.0 mg of CPA/kg of body weight, or with solvent alone (1.0 N sodium bicarbonate, 2.0 mL/kg). The birds were decapitated 3, 24, 48, or 96 h after dosing, and breast and thigh muscle was removed. The meat samples were stored at −80 °C until analyzed for CPA.

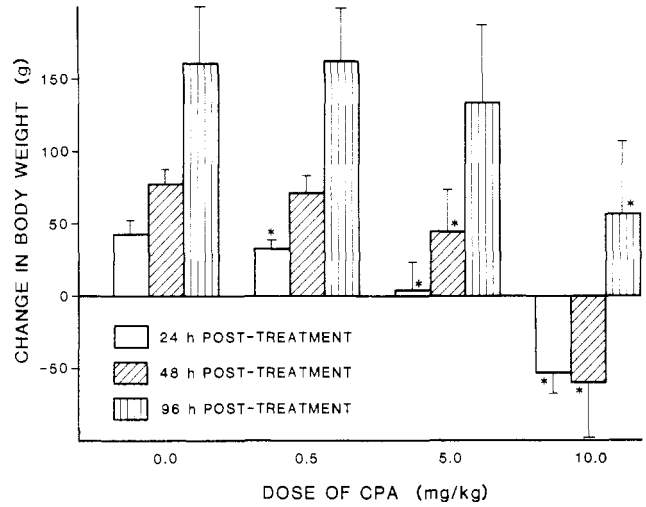

Figure 2. Effect of oral administration of single doses of cyclopiazonic acid on change in body weight. Values are means and standard deviations for 10 birds per dose–kill time group.

Analytical Methods. Meat samples were thawed and ground with a meat grinder, and 50-g samples were assayed for CPA by a ligand-exchange high-performance liquid chromatography (HPLC) method previously described (Norred et al., 1986). Briefly, the samples were extracted with chloroform–methanol (80:20), and interfering components of the extract were removed by partitioning into 0.1 N sodium hydroxide and by minicolumn chromatography. For HPLC analysis, a reversed-phase ODS column and a mobile phase containing 0.025% 4-dodecyldiethylenetriamine, 1.0 mM zinc acetate, and 1.0% ammonium acetate in water–2-propanol–acetonitrile (1:1.5:2) was used. Recovery of CPA was assumed to be 70%, based on analyses of spiked samples of meat. CPA was quantitated by comparison with HPLC peak areas of known amounts of authentic CPA. Samples of diet were ground in a Wiley mill, then extracted, and assayed for CPA by the same procedure as that used for meat. Data were analyzed with computerized procedures for analysis of variance or for correlation coefficients (SAS Institute, Inc., 1982).

RESULTS AND DISCUSSION

Birds given CPA had reduced weight gains (Figure 2), and the severity of the effect was dose dependent. Birds that received 10 mg of CPA/kg of body weight lost weight within 24 h. By 96 h after treatment they had begun to recover but were stunted compared to controls. Birds given 0.5 or 5.0 mg/kg gained weight, but at significantly slower rates than birds in the control group.

Figure 3 shows typical HPLC chromatograms of extracts obtained from breast and thigh muscle. Standards of CPA in methanol had retention times of ca. 7.0 min. Meat extracts from birds given solvent only had no peaks with retention times greater than 2–3 min. Samples from birds given CPA had symmetrical peaks with retention times that corresponded with that of CPA, and the area of these peaks was dependent on both dose of CPA and time after administration. Several peaks eluted from the column with retention times intermediate to that of the solvent front and CPA. These peaks may be metabolites of CPA, or

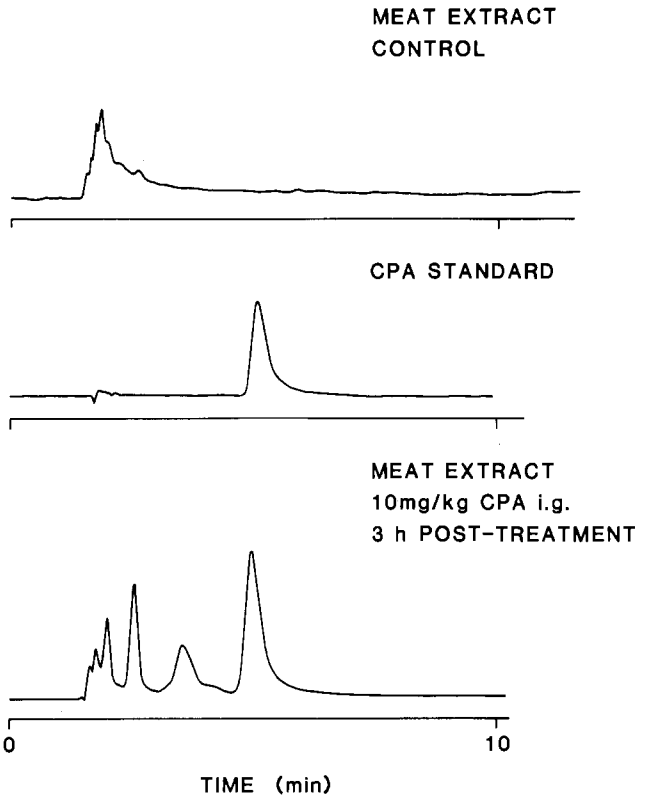

Figure 3. Chromatograms of meat extracts from birds given solvent only or 10 mg of CPA/kg of body weight intragastrically (ig) compared with chromatogram of CPA standard. Detector: UV at 284 nm.

Table I. Correlation of Weight Loss with CPA Content of Meat

time to sacrifice, h	dose of CPA, mg/kg		
	0.5	5.0	10.0[a]
24	0.216	−0.258	0.122
48	0.106	−0.396	−0.784**
96	−0.185	0.302	−0.543*

[a] Key: *, significant, $P < 0.1$; **, significant, $P < 0.01$.

possibly chelated forms of CPA with different chromatographic properties. Attempts to purify these components by preparative methods, including liquid chromatography and thin-layer chromatography, in quantities sufficient for identification have so far been unsuccessful.

The appearance of CPA in meat and its rate of disappearance is shown in Figure 4. The highest levels of CPA were found in muscle from birds given 10 mg of CPA/kg of body weight 3 h after treatment, with a mean of 2500 ppb, and the content in 2 of 10 birds in this treatment group exceeded 5000 ppb. For each dose level given, the maximum content of CPA in the meat was seen 3 h after administration, and for the 0.5 and 5.0 mg/kg treatment groups, the CPA was eliminated from the meat rapidly over the next 24 to 48 h. However, birds given 10 mg of CPA/kg of body weight retained a greater proportion of the dose in the meat, so that the rate of elimination was slower than that of the other treatment groups. Examination of the data indicated that the content of CPA in the meat of individual birds given 10 mg/kg was dependent on the amount of weight lost (or gained) by that particular bird. Table I shows a significant correlation between weight loss and amount of CPA in the meat 48 h after administration of 10 mg of CPA/kg of body weight, but not at the other dose levels or sacrifice times studied. This finding indicates that birds suffering the toxic effects of CPA had the greatest difficulty in eliminating the toxin

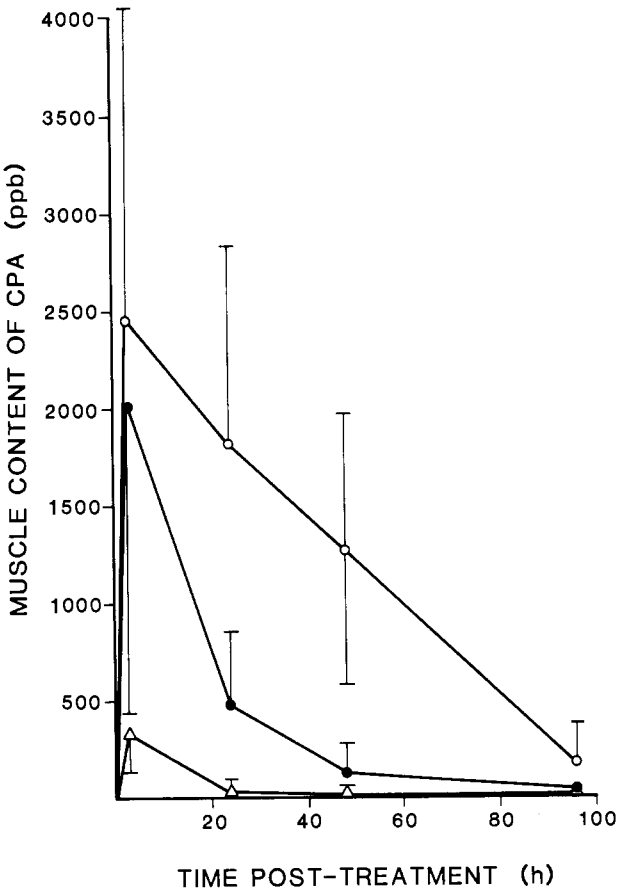

Figure 4. Content of CPA in meat of birds given single oral doses of CPA: 0.5 mg/kg (▲); 5.0 mg/kg (●); 10.0 mg/kg (○). Each point is the mean and standard deviation for 10 birds.

from meat and could mean that unthriftiness caused by CPA intoxication or by other toxins, such as aflatoxin, could result in greater amounts of CPA in edible tissues.

The occurrence of a mycotoxin in edible tissues is cause for concern for human consumers. The importance of this finding is dependent on the inherent toxicity of the mycotoxin and on the incidence and degree of contamination of the commodity. Since surveys of CPA in agricultural commodities have not been done, the question of the extent of the hazards posed by CPA cannot be answered. CPA is acutely toxic to many species, with the most sensitive models studied thus far being the dog (Nuehring et al., (1985) and the pig (Lomax et al., 1984). There is some disagreement in the literature on the toxicity of CPA in the rat. These differences are probably a result of differences in dosage regimen or route of administration (Purchase, 1971; van Rensburg, 1984; Morrissey et al., 1985; Hill et al., 1986). Since CPA accumulates to a significant degree in edible tissues, further studies on the effects of chronic exposure of animals and humans are indicated. Also the occurrence of CPA in corn and other foods and feed should be determined.

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Lysine Absorption from Chickpea (*Cicer arietinum*) and Milk-Supplemented Wheat Diets at Two Levels of Energy

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Nine young adult girls were fed diets containing wheat, wheat and chickpea, or wheat and milk at each of the two energy levels of 1600 and 1900 kcal/day. Lysine was estimated in food and feces. From a balance study, nitrogen balance, true digestibility (TD), biological value (BV), and net protein utilization (NPU) were calculated. Results indicated that lysine excretion was negatively correlated with lysine intake. Percent lysine absorption was significantly higher ($P < 0.01$) from high-energy diets. Percent lysine absorption was significantly related to dietary protein sources. At the lower, but not at the higher, level of energy consumption lysine absorption was significantly related to N balance, BV, and NPU of the diets.

Diets consumed in developing countries are predominantly cereal based, and lysine is usually the first limiting amino acid in these diets. The indispensable nature of lysine in human diets has been well established (Rose et al., 1954). In addition to protein synthesis for growth and maintenance, lysine plays an important role in several bodily functions (Jansen, 1962; Kurup et al., 1983).

The amino acid composition of a protein is usually studied to determine its protein quality. However, amino acid availability to the individual is also important. Since lysine is the first limiting amino acid of cereal-based diets, its absorption from common dietary regimes becomes all the more important. Many studies relating to effect of lysine supplementation on nitrogen balance improvement have been reported in the literature (Rice et al., 1970; Clark et al., 1962; Jones et al., 1956). However, absorption of lysine from different dietary regimes has not been well studied. A recent study of Meredith et al. (1986) showed that consumption of meals increased plasma lysine only when it was amply supplied by the diet and when subjects consumed less than 20 mg/kg per day decreased plasma

Table I. Characteristics of the Subjects

subject	wt, kg	ht, cm	age, years
AD	47.5	155.0	21
AN	41.5	156.3	22
BA	45.0	158.7	21
IN	50.0	165.0	21
KB	50.0	162.5	21
LA	48.0	156.2	21
ME	46.0	158.7	22
PS	46.0	152.5	22
VS	46.5	156.2	21

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