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Toxicological Evaluations of Cyclopiazonic Acid and Ochratoxin A in Broilers

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ABSTRACT The individual and combined effects of ochratoxin A (OA) and cyclopiazonic acid (CPA) were evaluated in Petersen × Hubbard broiler chickens from 1 d to 3 wk of age. The experimental design was a 2 × 2 factorial with treatments of 0 and 2.5 mg OA/kg feed and 0 and 34 mg CPA/kg feed. Production performance, serum biochemistry, and gross pathological observations were evaluated. Body weight gain was reduced (P < 0.05) by OA, CPA, and OA-CPA in combination at the end of 3 wk. Ochratoxin A significantly increased the relative weight of the kidney and serum concentrations of uric acid and triglycerides and decreased total protein, albumin, and cholesterol. The toxicity of CPA was expressed primarily through increased relative weights of the proventriculus and increased activity of creatine kinase. Exposure to OA-CPA was characterized by increased relative weights of the liver, kidney, pancreas, and proventriculus; decreased concentrations of serum albumin, total protein, and cholesterol; increased activity of creatine kinase; and increased concentrations of triglycerides and uric acid. Postmortem examination revealed that the chickens fed CPA or OA-CPA had thickened mucosa and dilated proventricular lumen. Data from this study demonstrate that OA, CPA, and the OA-CPA combination can limit broiler performance and adversely affect broiler health. The interaction of the compounds was primarily additive or less than additive in the parameter in which the interaction occurred.

(Key words: cyclopiazonic acid, ochratoxin A, broiler chick, organ weight, serum constituents)

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INTRODUCTION

Mycotoxins comprise a structurally diverse family of naturally occurring fungal-elaborated toxins, many of which have been implicated in disease outbreaks in humans and animals. The toxicity of mycotoxins in animals ranges from acute symptoms to chronic disease, death, and interference with reproductive efficiency (CAST, 1989). Cyclopiazonic acid (CPA) and ochratoxin A (OA) have been evaluated individually for their toxicities in chickens (Prior and Sisodia, 1978; Huff et al., 1979; Dorner et al., 1983; Porter et al., 1988; Smith et al., 1992). Cyclopiazonic acid is a mycotoxin that was first isolated from a culture of Penicillium cyclopium during a screening for toxigenic molds (Holzapfel, 1968); however, the mycotoxin is produced by several other fungal species of the genera Penicillium and Aspergillus, including Penicillium camemberti (Nishe et al., 1985). Cyclopiazonic acid producing fungi grow on a variety of substrates including peanuts (Lansden and Davidson, 1983), cheese (Le Bars, 1979), and corn (Gallagher et al., 1978). It has been reported to occur naturally in Aspergillus flavus-contaminated corn (Gallagher et al., 1978). Aspergillus flavus, a producer of CPA, is a major contaminant of corn and peanuts in the southern U.S. (Dorner et al., 1983). The toxic effects of CPA in poultry are well documented. There have been reports of hyperemia and ulceration of the proventriculus, focal necrosis in the liver and spleen, lymphoid depletion of the bursa of Fabricius, weight loss, and changes in relative organ weight and serum biochemistry values (Dorner et al., 1983; Kubena et al., 1983, 1988, 1989; Wilson et al., 1986; Cullen et al., 1988; Smith et al., 1992). Another family of mycotoxins produced by the Penicillium and Aspergillus genera are the ochratoxins. Ochratoxin A has been identified as the most potent of its family

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Abbreviation Key: CPA = cyclopiazonic acid; OA = ochratoxin A.
of compounds. Ochratoxin A has been found to occur in various grains, cereals, and other plant products, animal feeds, meats, and human tissues in countries throughout the world (Bauer and Gareis, 1987; Bacha et al., 1988; Bauer et al., 1988; Marquardt et al., 1988; Jelinek et al., 1989). Studies have shown that OA adversely affects the health of poultry (Bruckner, 1988a, b, c; Sreemannarayama et al., 1989; Smith et al., 1992) and that it is extremely toxic to pigs (Steyn, 1984) and dairy cattle (Wei et al., 1973). It is absorbed passively through the intestinal tract and in an active manner in the kidneys. It is subjected to intestinal secretion and reabsorption via enterohepatic recycling. Binding of OA to the albumin fraction in the blood and recycling in the bile and kidney contributes to its long life in animals (Marquardt and Frolich, 1992).

Although extant literature has not shown that OA and CPA occur simultaneously, exposure to these mycotoxins at the same time is very possible. This assumption is justified based on possible exposure to more than one toxin through the use of multiple grain sources. Furthermore, production of more than one of these toxins by species of Aspergillus and Penicillium has been documented (CAST, 1989). Based on potential simultaneous exposure, this study was conducted to determine the effect of OA and CPA singly and in combination on the growth of broilers.

**MATERIALS AND METHODS**

**Experimental Animals**

One-day-old male broiler chicks of Petersen x Hubbard strain were obtained from a commercial hatchery. They were reared in wire-floored brooding cages with a brooding temperature of 35 to 40 C, a room temperature of 22 to 25 C, continuous fluorescent illumination, and forced ventilation.

**Preparation and Administration of Ochratoxin A and Cyclopiazonic Acid**

Ochratoxin A was produced, extracted, and purified by methods previously described by Huff et al. (1974). It was determined to be greater than 95% pure using an OA authentic standard by reverse phase HPLC3. Ochratoxin A was incorporated into the basal diet by dissolving the toxin in 1N sodium bicarbonate and then mixing the appropriate quantities with 1 kg diet.

Cyclopiazonic acid was obtained from Sigma Chemical Company4. The purity of CPA was determined using HPLC and spectrophotometry and was found to be greater than 98% pure. Cyclopiazonic acid was incorporated into the basal diet by dissolving the toxin in 1N sodium bicarbonate and then mixing the appropriate quantities with 1 kg diet. After drying, the 1 kg diet containing the appropriate toxin was then mixed with the rest of the basal diet to produce the desired treatment containing OA, CPA, and OA-CPA. The basal diet was analyzed for other mycotoxins before feeding and was found not to contain detectable levels of aflatoxin, deoxynivalenol, zearalenone, ochratoxin, or CPA.

**Experimental Design**

The chicks were weighed, wing-banded, and randomly assigned to different treatment groups. Eighty day-old chickens were housed in 16 cages, (five chickens per cage). Treatments were replicated four times. The chickens were fed a commercial, unmedicated, corn-soybean meal basal diet that contained or exceeded the levels of critical nutrients recommended by the National Research Council (1984). Based on previously published data on OA (Gibson et al., 1990) and CPA (Smith et al., 1992), the following dietary treatments were selected for use in this experiment: 1) control basal diet containing no added toxins, 2) diet containing 2.5 mg OA/kg, 3) diet containing 34 mg CPA/kg, and 4) diet containing 2.5 mg OA and 34 mg CPA/kg feed. Water and feed were provided for ad libitum consumption. Parameters measured were weekly BW change and feed utilization, daily mortality, relative weights of selected organs, and serum biochemistry indicators of toxicity at the termination of the experiment (21 d). Serum biochemical endpoints were analyzed using a clinical chemistry analyzer5 according to the manufacturer’s recommendation.

**Statistical Analysis**

All data [n = 20, except feed conversion (n = 5)] were analyzed by an ANOVA (Ott, 1988) using the SAS GLM procedure for 2 x 2 factorial ANOVA (SAS Institute, 1982). Treatment means with significant differences were ranked by Duncan’s multiple range test (SAS Institute, 1982). All statements of differences were based on significance at P < 0.05.

**RESULTS AND DISCUSSION**

The possibility of exposure of poultry and livestock to various combinations of mycotoxins is of much concern and warrants investigation of the potential effects of possible combinations occurring naturally. Generally, the effects of exposure to several combinations of mycotoxins in poultry have been reported to be additive. However, synergistic interactions have been observed for aflatoxin and OA (Huff and Doerr, 1981; Huff et al., 1983) and aflatoxin and T-2 toxin (Huff et al., 1988b,c), and an antagonistic interaction has been observed for ochratoxin A and diacetoxyscirpenol (Kubena et al., 1994). The toxicity of CPA in combination with T-2 was studied in broiler

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3Waters HPLC system with HPLC pumps (Waters 501), autosampler (WISP 710B), SIM, computer program (Maxima 820), Waters Scientific, Milford, MA 01757.
4Sigma Chemical Co., St. Louis, MO 63178-9916.
5Gilford Impact, 400E, Ciba Corning Diagnostics Corp., Gilford Systems, Oberlin, OH 44774.
birds, and a synergistic interaction was indicated by the liver and kidney relative organ weights, whereas, in other endpoints, interactions were primarily additive or less than additive (Kubena et al., 1994). Results from a previous study in our laboratory revealed that CPA at 50 mg/kg interacted synergistically with aflatoxin at 3.5 mg/kg feed and adversely affected growth of treated birds (Smith et al., 1992).

Data representing the toxic effects of OA and CPA, singly and combined, in broiler birds are shown in Tables 1 to 3. The toxicity of OA and CPA was expressed as a reduction in BW gain and as changes in relative weights of the pancreas, kidney, proventriculus, and serum biochemistry. Cyclopiazonic acid had a significant effect on BW gain at the end of Week 1 (Table 1). Body weight of CPA-treated birds was approximately 11% less than that of controls. Although there was a nominal change in BW for OA, this was not significant, and the combination remained practically unchanged. Further expression of toxicity was observed by the end of Week 2 when the BW of OA and CPA birds singly were 19.3 and 17.6%, respectively. Interestingly, the combination of OA-CPA associated change in BW was noted at 31%. Feed conversion was not significantly affected by any of the treatment groups.

Ochratoxin A and CPA appear to have different mechanisms in their induction of toxicity; however, one common result of their action is inhibition of protein synthesis. Cyclopiazonic acid inhibits translocation by blocking a ribosomal site involved in peptide formation (Yates et al., 1987). Inhibition of translocation may ultimately result in the inhibition of synthesis of proteins that are vital for development, tissue repair, and proper functioning of organs (Smith et al., 1992). It is probably in this manner that CPA induces its toxic effect, which results in reduction of BW.

Several studies devoted to elucidating the molecular mechanism of OA toxicity indicate that the primary effects are disturbance of calcium homeostasis inherent to lipid peroxidation processes (Rahimtula et al., 1988; Rahimtula and Chong, 1991), inhibition of mitochondrial respiration (Fink-Gremmels et al., 1995), and inhibition of tRNA synthetase. Ochratoxin A also was thought to inhibit protein synthesis by competitively binding to phenylalanyl-tRNA synthetase. Recently in vitro studies indicated that L-phenylalanine does not reduce OA toxicity (Bruinink and Sidler, 1997; Bruinink et al., 1997). The primary assumption of these studies is that OA toxicity does not affect phenylalanine-dependent processes (Bruinink and Sidler, 1997). It is possible that OA does not inhibit phenylalanine-tRNA synthetase directly by competing with phenylalanine. Ochratoxin A, however, probably inhibits aminoacyl-tRNA formation indirectly by inhibiting adenosine triphosphate production by the mitochondria (Fink-Gremmels et al., 1995). Adenosine triphosphate is required to activate amino acids prior to association with their specific tRNA. Because OA and CPA can inhibit protein synthesis, it would be expected that simultaneous exposure would greatly retard growth rate and inhibit other physiological processes that are protein dependent.

Results of the influence of OA and CPA singly and in combination on relative organ weights are shown in Table

### Table 1. Influence of ochratoxin A (OA) and cyclopiazonic acid (CPA) (singly and combined) on body weights and feed conversion of broiler chickens

<table>
<thead>
<tr>
<th>OA (mg/kg)</th>
<th>CPA (g)</th>
<th>Day-old</th>
<th>Week 1</th>
<th>Change</th>
<th>Week</th>
<th>Change</th>
<th>Week 3</th>
<th>Change</th>
<th>Feed conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>44.9 ± 0.7a</td>
<td>96.2 ± 3.2a</td>
<td>49.3 ± 9.1a</td>
<td>674.5 ± 16.4a</td>
<td>1.6 ± 0.05a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>0</td>
<td>46.0 ± 0.6b</td>
<td>94.1 ± 2.2b</td>
<td>300.4 ± 7.5b</td>
<td>521.1 ± 19.4b</td>
<td>1.57 ± 0.07a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>34</td>
<td>44.6 ± 0.7a</td>
<td>85.7 ± 3.9b</td>
<td>-10.9</td>
<td>306.8 ± 15.9b</td>
<td>-17.6</td>
<td>556.3 ± 18.4b</td>
<td>-17.5</td>
<td>1.59 ± 0.03a</td>
</tr>
<tr>
<td>2.5</td>
<td>34</td>
<td>45.6 ± 0.7a</td>
<td>95.5 ± 3.1a</td>
<td>0.0</td>
<td>258.3 ± 10.4b</td>
<td>-30.6</td>
<td>441.1 ± 24.5b</td>
<td>-34.6</td>
<td>1.63 ± 0.10a</td>
</tr>
</tbody>
</table>

Values within columns with no common superscript differ (P < 0.05).

Values represent the mean ± SEM of four groups of five broilers per treatment less mortality.

Values represent interaction (P < 0.05).

### Table 2. Effects of ochratoxin A (OA) and cyclopiazonic acid (CPA) (singly and combined) on the relative weight of the liver, kidney, pancreas, and proventriculus

<table>
<thead>
<tr>
<th>OA (mg/kg)</th>
<th>CPA (g)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Proventriculus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>3.30 ± 0.09b</td>
<td>0.55 ± 0.01c</td>
<td>0.36 ± 0.01b</td>
<td>0.60 ± 0.02b</td>
</tr>
<tr>
<td>2.5</td>
<td>0</td>
<td>3.71 ± 0.17b</td>
<td>0.73 ± 0.07b</td>
<td>0.40 ± 0.02b</td>
<td>0.65 ± 0.03b</td>
</tr>
<tr>
<td>0</td>
<td>34</td>
<td>3.32 ± 0.13b</td>
<td>0.51 ± 0.02b</td>
<td>0.39 ± 0.02b</td>
<td>0.95 ± 0.07a</td>
</tr>
<tr>
<td>2.5</td>
<td>34</td>
<td>4.79 ± 0.21a</td>
<td>0.89 ± 0.08a</td>
<td>0.50 ± 0.03c</td>
<td>0.83 ± 0.04a</td>
</tr>
</tbody>
</table>

Values within columns with no common superscript differ (P < 0.05).

Values represent the mean ± SEM of four groups of five broilers per treatment less mortality.

Values represent interaction (P < 0.05).
TABLE 3. Effects of ochratoxin A (OA) and cyclopiazonic acid (CPA) (singly and combined) on serum total protein, albumin, uric acid, cholesterol levels triglycerides (Trig), creatine kinase (CK), and mean corpuscular volume (MCV)1

<table>
<thead>
<tr>
<th>OA</th>
<th>CPA</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Uric acid</th>
<th>Cholesterol</th>
<th>Trig</th>
<th>CK</th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg</td>
<td>g/100 ml</td>
<td>mg/100 ml</td>
<td>mg/dL</td>
<td>IU/L</td>
<td>µm3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2.69 ± 0.06a</td>
<td>1.21 ± 0.03a</td>
<td>5.56 ± 0.42b</td>
<td>170 ± 4.1a</td>
<td>70 ± 8b</td>
<td>786 ± 80a</td>
<td>150.8 ± 1.9a</td>
</tr>
<tr>
<td>2.5</td>
<td>34</td>
<td>2.64 ± 0.07a</td>
<td>1.11 ± 0.03ab</td>
<td>6.57 ± 0.51a</td>
<td>172 ± 6.8a</td>
<td>69 ± 4a</td>
<td>1,220 ± 204b</td>
<td>153.4 ± 2.07a</td>
</tr>
<tr>
<td>2.5</td>
<td>34</td>
<td>1.88 ± 0.11a</td>
<td>0.80 ± 0.06c</td>
<td>14.46 ± 1.53b</td>
<td>117 ± 7.4a</td>
<td>116 ± 8a</td>
<td>2,167 ± 445b</td>
<td>146.6 ± 2.5b</td>
</tr>
</tbody>
</table>

1Values represent the ± SEM of four groups of five broilers per treatment less mortality.

2. Ochratoxin A alone increased the relative weight of the kidney, whereas CPA alone increased the relative weight of the proventriculus. These results are consistent with those of Huff et al. (1974, 1988a). The OA-CPA combination treatment increased the relative weight of the proventriculus, pancreas, kidney, and liver. There was a significant interaction between the mycotoxins, indicated as increase in the relative weight of the liver.

The deleterious effect observed in kidneys of OA-CPA treated birds is assumed to be the result of OA nephrotoxicity (Elling et al., 1975). Chronic exposure of chickens to OA has been associated with decreased urine concentration, decreased glomerular filtration, decreased phenol clearance, and impairment of proximal tubular function (Huff et al., 1975). Furthermore, Glahn (1993) reported that OA-treated birds were observed with degeneration and structural alterations in the renal tubular epithelium, with the most severe effects occurring in the proximal tubules.

Results of serum biochemical analyses are presented in Table 3. Cyclopiazonic acid had no significant effect on serum total protein, albumin, uric acid, cholesterol, or triglyceride levels. Similar observations of total protein, albumin, cholesterol, and triglycerides levels in serum were made by Smith et al. (1992) for CPA at 50 g/kg feed; however, in their study, serum uric acid level increased significantly. A possible reason for the discrepancy between the two results is the higher concentration of CPA toxicity because CPA alone increased creatine kinase activity but OA had no effect.

The induction of toxicity observed following exposure to OA alone and CPA alone indicates that these two mycotoxins express their toxicity by different mechanisms. The results also indicate that simultaneous exposure to two mycotoxins may exacerbate the toxic effect above each one alone. The effect resulting from interaction of OA and CPA in combination was additive, primarily in the parameter in which the interaction occurred. It should be noted that this effect may be organ-specific, for example, in this study, the liver.

REFERENCES


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